

The Biggest Lame Duck of All

THE British government has surprised even coal miners, not to mention the National Coal Board, with the munificence of the financial assistance it now proposes for the British coal industry in the next five years. In the House of Commons last week, Mr Peter Walker, the new Secretary of State for Trade and Industry, announced that his department will do virtually everything asked of it last May, when the National Coal Board and the National Union of Mineworkers ganged up to ask for a programme of financial assistance calculated to keep the coal industry in being, at its present rate of production of about 130 million tons a year, for a decade or more. Only on the question of whether imports of coal into Britain should be restricted have the coal board and the mineworkers been denied, and it is hard to see how the government could have behaved otherwise with British membership of the European Steel and Coal Community just around the corner. Briefly, it is proposed, in the Coal Bill now published, that £475 million should be written off the coal board's accumulated debt and deficient, also that the coal board should be allowed to borrow up to £550 million for future requirements (which may later be increased to £700 million), that the government should provide the board with 50 per cent of the cost of redeploying men and closing down collieries, that the government should finance a more equitable redundancy scheme for coal workers and also help with better pensions, that it should continue to subsidize the burning of coal in the power stations where this is not competitive and that the coal industry should benefit from the policy of grants to economically depressed regions of Britain at a cost which may amount to more than £200 million in the next five years. Several questions arise, not the least of which is whether the present government has followed its predecessors in the abandonment of economic yardsticks in the making of a policy for fuel.

The central issue is whether it is wise—which is not the same as to whether it is politically expedient—to embark on a policy of cheap coal at this stage in the economic history of Britain. In the next five years, the total cost of the measures announced last week could well exceed £1,000 million, the equivalent of more than £1.50 for each ton of coal produced or between 20 and 25 per cent of the turnover of the nationalized coal industry from the sale of coal. It is not clear, from what Mr Walker said in the House of Commons last week, whether it has been possible to extract from the National Union of Mineworkers an undertaking that the impending claim for higher wages will be modest, but the chances are not especially bright. Ironically, however, the past few months have seen a natural change in the economic competitiveness of coal which might by itself have provided the kind of security for which the National Union of Mineworkers and the coal board have been asking. In the past two years, since July 1970, the wholesale price of heavy fuel oil of the kind used in power stations has increased by no less than 28 per cent. Since the end

of 1970, the price of crude oil in Kuwait has increased from \$1.68 a barrel to \$2.37 a barrel, at which price, at the thermal equivalent of 7.3 barrels of oil to 1.5 tons of coal, even the chief source of crude oil in Kuwait, innocent of transport and refining charges, is now more than 80 per cent of the price of the thermal equivalent in coal of the average quality sold by the National Coal Board, and probably exactly the same price as the coal supplied on bulk contracts to the Central Electricity Generating Board. In the circumstances, might it not have been just as prudent for the government to refrain from taking measures the effect of which will be to absolve the National Coal Board from the kinds of practices to which other nationalized industries are subjected but instead to let coal find its own level relative to oil?

The issue is important for two reasons. First, although the National Coal Board is a special case and has particular problems to contend with in dealing with the social problems which arise when geographical shifts, if not much further contraction, are inevitable, but it is the kind of special case likely to set daunting precedents for other industries. And it goes without saying that the coal industry and the government have not yet replied convincingly to the kinds of criticisms raised by the Select Committee on the Nationalized Industries in 1968, which specially complained of the way in which the coal industry kept its own rules on depreciation policy. If the National Coal Board had been made to toe the common line at that date, Mr Walker's handout would now be much smaller. But a further measure which helps to make still more nonsensical the relationships between the several nationalized industries may be reckoned, at the very least, to lead others to believe that financial rules on depreciation and the proper return on capital apply only to successful industries.

The second reason why an artificial reduction of the price of coal may be unwise, at this stage in the development of the energy policy of Western Europe, is that this step will diminish the incentives which should now be sought for economizing in the use of fuel of all kinds. It is true, of course, that for much of manufacturing industry, fuel costs are usually a small proportion of the total, so that quite large percentage savings in the use of coal and other fuels are often economically unimportant, but it is hard to see how industrial enterprises can be harried along in the right direction if they are not somehow helped to appreciate the full costs involved. Both in the United States and in Western Europe, there is increasing anxiety about the extent to which imports of petroleum in the years ahead will strain the collective balance of payments—imports of petroleum into Western Europe are reckoned to increase from 613 million tonnes in 1970 to more than 1,000 million tonnes in 1985 and long before then the European Community will no doubt be thinking of ways of moderating this burden, most probably by means of import duties. In other words, the price of oil in Europe is likely to increase even more rapidly than



the wholesale price in the Middle East. Unnaturally cheap coal in the later years of this decade will in other words still further distort the pattern of energy consumption. Yet energy is so rudimentary, and the resources employed in its provision are so vast, that a distortion of the natural economic pattern can mean a great wastage. Why, in the circumstances, has a Conservative government chosen, for at least the next five years, to override the market?

The package of proposals announced last week by Mr Walker also raises important questions about the arrangements that should be made to deal with industrial change and the social consequences thereof. It is of course entirely proper that a major industry such as the coal industry should do what it can to keep its labour force intact and cheerful and that it should have schemes for helping men to move from one place to another as old coal fields die out and new ones are developed. But should not the costs of such a policy fall directly on the employer and not the central government? And in circumstances when it turns out that unwanted miners must be laid off, is it not much more appropriate that the government should make sure that its arrangements for dealing with the social consequences of industrial change apply equally to mining communities and to others? One of the unhappy features of Mr Walker's proposals is that it has singled out the miners for special treatment. It would have been much better if the government had recognized its obligation to provide fair and generous arrangements for everybody.

To the extent that the assistance now being provided for the British coal industry will in due course determine the trend of energy consumption in Britain and, by extension, in Western Europe as a whole (for the coal board has clearly set its heart on an export trade once British membership of the European Community is a fact), it is also fair to ask why the new proposals have not been accompanied by some policy on research. For all its anxiety about the future, the National Coal Board makes only modest investments in research and development, chiefly in the processing of coal and the development of new mining techniques. But if coal is to become a crucial part of the British fuel economy, is it not now necessary that the coal board should also invest in the kinds of enterprises that would make easier the exploitation of coal seams which are at present uneconomic? Underground gasification is one obvious possibility to which very little thought has been given in Britain during the past decade. But it would also be prudent that the coal board should revive some of the interest of past decades in the manufacture of liquid fuels from solid coal and the conversion of solid fuel into more usable kinds of gas. In short, there are the strongest possible reasons why the financial help which the National Coal Board has now been promised should be accompanied by a firm instruction on its conduct of research and development. The scale of the research programme should be increased, and its direction should be altered in such a way that the coal board will be able to make its proper contribution to the development of a constructive policy in the years ahead. But there is also a case for reexamining the arrangements for research and development throughout the fuel industries. Mr. Walker might well ask why the new arrangements for industrial research at the Department of Trade and Industry say nothing about fuel.

Squeeze on Postgraduates

THE reaction of the universities, like that of other British education institutions, to Mrs Margaret Thatcher's white paper on education (see *Nature*, 240, 372; 1972) has been every bit as generous as she can have hoped. The plan to make nursery school places available for all who want them and to improve staffing ratios in schools is recognized, even by those adversely affected by the general balance of the new education programme, to be desirable. But too much adulation should not conceal the serious effects which Mrs Thatcher's programme will have on the development of postgraduate studies at British universities. In the academic year just finished, the number of postgraduate students was the equivalent of 45,000, roughly 19% of the effective number of full-time students at British universities. The intention is that the number of postgraduate students should rise by 7,000 in the new quinquennium but less quickly than the increase in the university population as a whole so that, in 1976-77, postgraduates will account for 17% of the total full-time student population. What this implies is that the expansion of postgraduate studies at British universities will be less than two-thirds of what it would have been if the ratio now established had been held constant. It is in fact only a little more than 40% of what the universities had asked for. Because it is inevitable that a large part of the increase now planned will be taken up by an expansion of such fields as social, administrative and business studies, it follows that the new plan will be especially harmful in engineering, technology and science. The consequences are certain to be sufficiently serious to change the character of academic work in many university departments.

In all the circumstances, there is good reason why British universities should now urgently reassess the part played by postgraduate studies in their academic work. And it remains true that postgraduate teaching, especially that which involves research, is intrinsically a part of the work of scholarship which is, in the last resort, the chief reason for the continuing existence of the universities. Unless university teachers can continue to rely on the collaboration of young people with new ideas and skills, they will themselves be less able and innovative than they might be. So much is beyond dispute and it is also the case that the kind of training provided by postgraduate courses of all kinds, those based on instruction and those involving research alike, is indispensable in the provision of the kinds of skills which all countries need in some degree—who, for example, will say that Britain could at this stage dispense with the activities of the business schools, most of which involve postgraduate degrees and diplomas? The practical question is not whether postgraduate teaching is necessary but that of knowing how much there should be of it and how it should be distributed among the several universities and the different departments within them, given the diminished expansion now in prospect. These are the questions to which the University Grants Committee and the universities themselves will have to pay attention in the weeks ahead.

How should quantitative yardsticks be constructed? The first thing to say is that the need to maintain the supply of teachers in higher education, especially at the

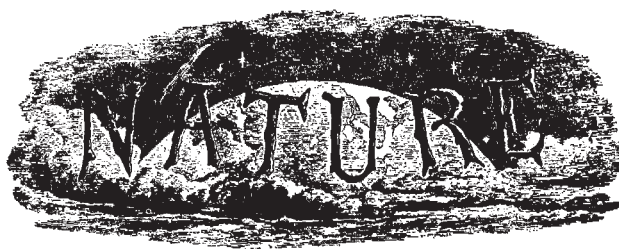
universities and the polytechnics, should provide some kind of lower limit to the scale on which postgraduate studies are undertaken. In 1970, there were close on 30,000 academics at British universities and, if Mrs Thatcher's plan that the polytechnics should have as many students as the universities by 1975 does eventually mature, there will be more than 60,000 teachers in higher education five years from now. On the conservative assumption that academics stay in higher education for twelve years on the average, this implies an annual recruitment of people with postgraduate experience of some kind into higher education which may eventually settle down at 6,000 a year but which should certainly be greater while the polytechnics are growing in size and also improving in quality. It may be true that the polytechnics will not wish to recruit orthodox PhD students exclusively to their staffs, but even by the narrow test of whether the proposed scale of postgraduate teaching is enough to cater adequately for teaching vacancies in higher education, the bottom of the barrel is uncomfortably close. In recent years, British universities have been turning out fewer than 5,000 doctorates each year (and the proportion of women is scandalously low, less than 10%). Although the Council for National Academic Awards has done well in setting standards for first degrees, it has so far made only a modest contribution to postgraduate studies. Because it is desirable that postgraduate courses should not be exclusively preparations for teaching in higher education, there is a danger that the scale of postgraduate training will not be enough to keep the universities and polytechnics properly supplied with teachers, especially when the large numbers of academics recruited to the universities during the rapid expansion of the 1960s begin to drift away into more secular occupations. This threat may seem remote just now, when the demand for academic jobs appears far bigger than the supply, but it would have been comforting if Mrs Thatcher's somewhat arbitrary decision that the needs of the universities should be scaled down had been backed up with at least an assurance that this problem had been considered.

Within the scope of what is now intended, the universities will have to make some fierce and often unpalatable decisions. Whatever may be said about the need that all university teachers should have an equal chance of attracting postgraduate students to work with them, there is at present some evidence that postgraduate studentships are scattered too thinly among British universities. The statistics published by the Science Research Council, the chief source of maintenance grants for research students in Britain, show that in fields such as astronomy and mathematics, several university departments have only one or two studentships in their gift, and the chances are that these are precisely the departments in which other funds are not available. However gifted the teachers concerned may be, however, postgraduate teaching on this kind of scale is bound to be unsatisfactory, at least to the extent that young men and women are not provided with the chance of seeing how others learn and of being stimulated by their contemporaries. Unhappily for the universities, however, the only long-term remedy for this situation is a still further rationalization of the teaching pattern within the university system. In recent years, the University Grants Committee has not been nearly as successful as it would have wished

in the encouragement of what the Science Research Council calls selectivity and concentration. The need is now, however, much more urgent than it has ever been and, moreover, the years ahead will demonstrate all too clearly to those who teach in higher education that the attrition of small departments will be one of the most effective ways of taking the edge off the more stringent staffing ratios now in prospect. In other words, it is in everybody's interest that there should now be a serious attempt further to rationalize the pattern of university teaching at all levels.

A further difficulty requiring study stems from the fact that no fewer than 27% of the postgraduate students in British universities are at present from overseas. Nobody will deny the value of international links like these and it is also true that a large proportion of the foreign postgraduate students at British universities are following comparatively brief courses. Yet there is also some evidence that the potential supply of postgraduate students from Britain is artificially restricted by the lack of financial support. This argues, at the very least, that the research councils should be provided, in the years ahead, with extra funds sufficient at least to support the extra 7,000 postgraduate students included in Mrs Thatcher's programme. Whether the time has come to reopen the thorny question of loans for postgraduate students is another matter. But it is also essential that extra funds for financing Mrs Thatcher's modest expansion should not be quarried from the general science budget, already too much under pressure.

100 Years Ago



The Meteorology of the Future

I CANNOT quite agree with Mr. Lockyer that the most important question in meteorology is the discovery of a cycle. Were it even so well proved, it would still be but an empirical law. In my opinion the chief desideratum of the science is a dynamical theory of barometric waves; and the data for this are to be found not merely in records of barometric fluctuations in one place, nor by comparing the records in several places at or near the sea level, but by comparing the records at places separated by the greatest possible vertical distance, though horizontally near each other. Such records do not yet exist, and they can be had only at specially chosen stations; the summit and the base of Teneriffe, for instance, or of Etna. The latter would probably be the best, as it is in the variables. It is not at all certain that the fluctuations of the barometer at the summit and at the base of a high mountain would be nearly alike. It is stated by Kaenitz that while the barometer in the hottest part of the day falls at the sea level, it rises at a height of a few thousand feet. The reason why it rises at the higher station is that the entire column of air is lifted up by the expansion due to heat, and thus a larger proportion of the column comes to be above the station. This cause does not act at the sea-level, and the barometer there falls in consequence of the outflow of air from the top of the column. It is much to be desired that the attention of scientific men and scientific committees should be directed to this subject, as without such sets of comparative observations we shall never have all the data for a complete theory of barometric waves.

Old Forge, Dunmurry

JOSEPH JOHN MURPHY

From *Nature*, 7, 142, December 26, 1872.

OLD WORLD

Contingency Plans Revealed by CNES

from La Recherche.

THE French unveiled plans this week designed to save the crumbling ELDO partnership. The reluctance of Germany to participate in the Europa III project has caused France to come out with details of a cheaper and, it claims, a more reliable launcher.

This move has been precipitated by the German preference to take part in building the exit module for the space shuttle which, in French eyes, implies that they are moving farther away from the concept of a European launcher.

France is well known to be reluctant to base a European space programme on launchers obtained from the United States and recently both Mr Michel Debré and Mr Charbonnel, Minister for Industrial and Scientific Development, have come out strongly in favour of a French national launching programme.

Details of the French plan, presented this week to the conference of European Ministers for Research, were first revealed at an international congress organized by the Centre National d'Etudes Spatiales (CNES) at Kourou in French Guiana at the end of November.

The proposed replacement is a three stage rocket in contrast with the two stage Europa III. France claims that this launcher can provide the same performance as Europa III but at a cost of Fr 2,200 million, compared with the estimated Fr 3,000 million which it would cost to develop Europa III.

The French government proposes that it should meet 75 per cent of the cost of the new launcher—Fr 1,700 million, which is Fr 300 million more than it would have contributed to Europa III. The extra cost would be carried by the French Ministry for the Army which sees an autonomous European launcher as a basis for launching observation and navigation satellites.

The first stage of the three stage launcher will be the same as that which is planned to form the first stage of Europa III—four Viking 2 engines developed by the Société Européenne de Propulsion. The second stage is to have one Viking 2 engine but the last stage will have a single cryogenic engine also developed in France. French project managers claim that this combination will be much more reliable than the Europa III launcher—particularly as the proposed second stage of Europa III consists of a complex cryogenic high

pressure engine which, say the French, is far from reliable. This, according to the French, should assure the German politicians, who are particularly concerned with the disastrous failures of Europa launchers, that their proposed launcher is based on well proven technologies.

Naturally enough France wants to have overall responsibility for the launcher—only 25 per cent of the cost is to be shared among her partners in ELDO—but it is hoped that European industry will contribute to its construction.

A question which is certain to be asked before decisions are taken on

the French plan is what use Europe will make of a launcher in the next twenty years. Last July, Germany, Britain and France made a survey of their scientific needs during 1980–90, and the remarkably consistent conclusions of each country are that between 40 and 50 launches will be needed in this decade.

France has argued that if European space plans were based entirely on launchers from the United States then only half this number of launches might be possible. Germany, on the other hand, feels that more than thirty of the launches would go ahead with United States launchers.

WOLFSON FOUNDATION

Train Research

THE Wolfson Foundation is to make grants worth about £1.25 million to support sixteen more university projects orientated to the needs of industry. This brings the total amount of money spent by the foundation in this way to about £3 million, and since the scheme started in 1968 forty projects have been funded (see *Nature*, 231, 75; 1971).

Prominent among the most recent awards are two concerned with research into magnetically levitated trains. The institutions concerned are the University of Warwick, where £147,750 is to be made available over the next five years to the Department of Engineering, and the School of Applied Sciences of the University of Sussex, which is to receive £127,655 over three years. The work at both these universities is naturally complemented by that in progress at the University of Wales Institute of Science and Technology (UWIST) where a centre for the technology of soft magnetic materials and their applications was set up two years ago with £132,000 from the Wolfson Foundation.

At Warwick it is planned to build a test vehicle that will be levitated above an aluminium track by means of superconducting magnets and propelled by a linear motor, whereas at Sussex conventional electromagnets and an iron rail on the track are to be used in a system involving magnetic attraction and a feedback control loop. And at UWIST a system based on the repulsive force between permanent magnets on the vehicle and on the track is being developed.

One of the advantages of using superconducting magnets is that they are lighter than their conventional

counterparts and allow clearances between vehicle and track of 25 cm or so, compared with the 1 cm or so that is possible with conventional magnetic methods. Even the air cushioned vehicle being developed by Tracked Hovercraft Limited, the protégé of the National Research Development Corporation, can hover only a few centimetres above its track.

Professor J. A. Shercliff, head of the School of Engineering Science at the University of Warwick, said last week that the work there will be directed towards a train capable of travelling at 300 to 500 miles an hour. He also said that the relatively large clearance possible between vehicle and track means that the tolerance in construction of the track will probably not need to be much greater than that of a normal road. Dr R. G. Rhodes, who is directly concerned with the Warwick project, points out that the Japanese have in the past few weeks announced the successful levitation of a vehicle weighing 4.5 tons using superconducting magnets. Much more government support for research into magnetically levitated trains is urgently needed, according to Dr Rhodes, otherwise the systems being developed by German companies (for example, Siemens) with government support, may win the day unchallenged.

Among the other projects which are to be financed by the Wolfson Foundation from the beginning of next year are the establishment of a unit at the University of Edinburgh for high speed liquid chromatography (£92,500), the setting up of an Institute of Offshore Engineering at Heriot-Watt University (£177,790) and the financing, for a period of five years, of a Chair of Energetics at the University of Newcastle upon Tyne (£37,500).

SOVIET SCIENCE

Human Rights ?

from our Soviet Correspondent

THE confiscation, last week, of the passport of Soviet physicist Valerii Nikolaevich Chalidze, while on a lecture tour of the United States, indicates a considerable threat to any future Soviet "liberal intellectuals" permitted to travel abroad. As Dr Zhores Medvedev points out, in his samizdat (underground) essay *Fruitful Meetings between Scientists of the World*, representatives of the Soviet Union, the technically independent delegations of the Ukrainian and Byelorussian Soviet Republics and five "countries of national democracies" were the only delegates which did not vote in favour of the UN Declaration of Human Rights. So presumably they do not consider themselves bound by Article 13.2 which states "Everyone has the right to leave any country, including his own, and to return to his country".

Valerii Chalidze was one of the three founder members of the illegal Human Rights Committee in 1970, whose aims were to study problems of rights and to help the Soviet authorities to introduce desirable reforms. He has been active, over the past few years, in contributing to the samizdat *Chronicle of Current Events* on matters of human rights and freedom of expression. Possibly on account of these activities, he was dismissed, in autumn 1970, from his position as head of the polymer physics school at the Moscow Institute of Plastics Research.

The Human Rights Committee has always insisted that it has no political aims and has constantly endeavoured (albeit unsuccessfully) to have itself registered as an official organization under Soviet law. Mr Chalidze, as secretary of the committee, has always preserved an attitude of great caution and discretion, so that this latest move may be construed as the pragmatically most effective means of neutralizing his protests. Earlier this year, the mathematician Alexander Esenin-Volpin was pressurized into emigrating "as a Jew" on the understanding that, if he did not emigrate, he would be either imprisoned or confined again in a mental hospital. Previous occasions when Esenin-Volpin was "hospitalized" as a dissident led to considerable public protest from Soviet intellectuals, so that in spite of the general policy of hindering Jewish intellectuals from leaving the country, the Soviet government apparently found it expedient, in this case, to urge him to leave. In fact, Esenin-Volpin never went to Israel at all, and is now teaching in New York.

In accordance with his previous stand for human rights, it is understood that Mr Chalidze will appeal against the

deprivation of his Soviet citizenship although he can have little hope for success. This latest move will leave physicist Andrei D. Sakharov the only notable member of the Human Rights Committee still active.

BROADCASTING

Sir Michael Moves On

SIR MICHAEL SWANN, principal and vice-chancellor of the University of Edinburgh, will succeed Lord Hill as chairman of the British Broadcasting Corporation on January 1, 1973. The announcement, made last week, will still speculation that Sir Michael was to succeed Sir Brian Flowers as chairman of the Science Research Council.

Sir Michael, who is 52, has been at the University of Edinburgh since 1952 when he was elected to the chair of Natural History at the university. Since 1965 he has been principal, and he was knighted earlier this year. During his time at Edinburgh Sir Michael has served on numerous public committees. He was a member of the now defunct Council for Scientific Policy from 1965-69, a member of the Medical Research Council from 1962-65, chairman of the Nuffield Foundation Biology project from 1962-65 and since 1969 he has been a member of the

Science Research Council. A spokesman for the council said last week that there was nothing in the SRC's charter to prevent Sir Michael from continuing to serve on the council.

Perhaps Sir Michael is better known for three other committees of which he was chairman. One report was on manpower resources, which pointed out that sixth form students were turning away from science, another considered whether it was advisable for Britain to partake in the 300 GeV accelerator project now under construction in Geneva, and a third advised on the use of antibiotics in animal husbandry and veterinary medicine. At present Sir Michael is a member of a committee looking into the veterinary profession.

The chairman of the BBC is expected to devote only part of his time to the job. The University of Edinburgh said last week that Sir Michael will have limited involvement with the corporation to start with, and he will still be active in the university until the end of the 1972-73 academic year. He will then sever connexions with the university and devote more time to the corporation. The chairman of the BBC normally holds office for five years, but as the corporation's charter expires in July 1976, Sir Michael has been formally appointed only until then.

SCOTLAND

Row over Gas Terminal

PROFESSOR V. C. WYNNE EDWARDS, former chairman of the Natural Environment Research Council is one of a distinguished group of academics and conservationists who are seriously concerned over joint proposals from the Gas Council and the Total Oil Company to build a gas terminal at Crimond airport between Fraserburgh and Peterhead north of Aberdeen. Only two weeks' notice has been given for objections to the scheme to reach Aberdeen County Council planning committee.

Crimond, a disused airfield, is situated by Loch Strathbeg near Rattray Head. The loch is the largest dune slack pool in Great Britain and is classified as being of exceptional scientific interest by the Nature Conservancy on three counts—wildlife, coastal geology and limnology.

In a letter to *The Scotsman*, Professor Wynne Edwards, who has the chair of Natural History at the University of Aberdeen, and G. M. Dunnet, Professor of Zoology at the university, make it plain that nothing like enough notice has been given of the proposal which is coming up for consideration by the planning committee today. The application is for outline permission only and the professors state that "the threat . . . cannot be assessed unless

detailed plans are provided. It seems to us quite incredible that an application for planning permission in principle, without details of the proposals, can even be considered when so much is at stake".

Dr R. Goodyear of the Scottish Nature Conservancy said last week that the loch "is an outstanding example of a northern dune system" which is important as a wildlife sanctuary and as an unusual example of mixed brackish and freshwater biology. According to the Royal Society for the Protection of Birds, which is adamantly opposed to the scheme, 5 per cent of the world's pink-footed goose population winter on the loch which is also an exceptionally important staging post for whopper swans on their way south from the breeding grounds in Iceland and the Soviet Union. Many thousands of ducks, swans and geese populate the loch.

Professor Dunnet last week emphasized that not only is he concerned about the possible damage to the site but also about the way the matter is being handled. He suspects that there is a lack of any real planning behind the proposal and says that there is no evidence that other sites have in fact been considered. Professor Dunnet is also afraid that other industry will be attracted to the area once the terminal is built.

NEW WORLD

Obesity, Public Health and Public Policy

by our Washington Correspondent

As examples of sheer quackery, non-prescription drugs and other devices designed to help people lose weight are hard to beat. Consider, for example, the product which is supposed to depress appetite, but which consists solely of corn syrup, vegetable oils, sweetened condensed whole milk and vitamins. According to Dr Jean Meyer, professor of nutrition at Harvard University, so-called slimming aids sold over the counter "are a gigantic fraud perpetrated on the American consumer . . . I have never seen any demonstration that any of them has any value whatsoever".

Dr Meyer's statement came during hearings on anti-obesity drugs conducted last week by the Senate Monopolies Subcommittee, and it was reiterated by every other witness before the committee. So much for the efficacy of non-prescription drugs which together account for a \$17 million a year market.

These products constitute a problem which is fairly easy to resolve, however, since they will come under an extensive review of the efficacy of all over-the-counter drugs which is now being conducted by the Food and Drug Administration, and action can be taken against those which fail to live up to the extravagant claims made for them. But what of the drugs which can only be obtained on prescription? They are an entirely different problem, and their regulation brings up important questions of public health and regulatory policy.

First, it must be borne in mind that the control of obesity is big business in the United States—there are about 30 million Americans between the ages of 21 and 65 who are believed to be at least 20 per cent overweight, and last year 26 million prescriptions were filled or refilled for weight-reducing drugs. One FDA official estimated last week that sales of such drugs amounted to about \$65 million last year alone. Second, obesity is an extremely complex complaint, whose causes range from the psychological and physiological to lack of exercise or simply overeating. But, as Dr Meyer pointed out, the average American is provided with the ideal atmosphere in which to become obese—labour-saving devices and personal transportation have reduced exercise, and there is almost unlimited access to food of all kinds.

Apart from its contribution to the

profits of drug companies, however, obesity is also a serious public health problem. It was suggested during the committee hearings last week, for example, that although expenditure on medical services has increased from about \$12,000 million in 1915 to about \$75,000 million this year, there has been no improvement in the life expectancy of the average American at the age of 20. The chief reason for this is the increased incidence of cardiovascular disease, in which dietary habits and obesity are important factors.

Because of the extent of obesity in the United States, and its relationship to public health, the Food and Drug Administration earlier this year initiated a thorough review of the efficacy of the prescription drugs that are now being used to treat obesity. The drugs, which are all designed to suppress appetite, are mostly amphetamines or related compounds, and there are about seventy different brands on the market. But a cost-benefit analysis of anti-obesity drugs is, however, complicated by the fact that since they nearly all stimulate the central nervous system, they have been widely abused and have a potential for drug dependency. This, of course, add to the risks which must be incorporated in the cost-benefit equation.

The central part of the FDA review was a series of 200 double-blind trials carried out on almost 10,000 patients, who were given either appetite-suppressant drugs or placebos. The results of these trials were reviewed by an independent panel of doctors led by Dr Thaddeus E. Prout, professor of medicine at Johns Hopkins University, and they were outlined at the committee hearings last week by Dr Henry E. Simmons, director of the Bureau of Drugs at the FDA.

In short, the review panel found that patients treated with appetite-suppressant drugs did lose more weight than those treated with placebos, but that the extra weight loss was "trivial"—no more than a fraction of a pound a week on average. It was found, however, that the extent of weight loss differed from trial to trial, and the increased weight loss in some trials "seems to be related to variables other than the drug prescribed", the panel noted. The panel also said that there was no evidence in the data from the trials to show that the combination of an appetite-suppressant with other drugs increases the benefits or reduces the risks.

On the strength of these results, Dr Simmons told the committee that the FDA is about to regulate the use of anti-obesity drugs more tightly. The first step will be a warning to doctors, published this week in the FDA Drug Bulletin which goes to every doctor in the country, that appetite-suppressant drugs have limited effect, and that they should only be used when other methods have failed. The FDA has also proposed labelling on the package to that effect. Finally, and perhaps most

BUDGETS

A Suit to Watch

by our Washington Correspondent

A LAW suit which has important implications in several areas, including some in which science and technology are involved, was filed last week in the Federal Court for the District of Columbia. Lawyers acting for the City of New York have charged that the Administration acted illegally in withholding \$6,000 million of the \$11,000 million that Congress had voted to give the states for construction of water pollution control facilities. The issue is whether the White House can legally withhold funds which have been appropriated by Congress—the Administration is expected to withhold funds earmarked for several other projects this fiscal year (see *Nature*, **240**, 374; 1972).

The White House has, in the past, based its authority to withhold funds on a law passed in 1870 which requires the Office of Management and Budget to sanction the expenditure of money by federal agencies, even though the money has already been appropriated by Congress. The courts have, however, not yet ruled on the constitutionality of withholding such funds, although a similar case has been filed by the state of Missouri claiming that the Administration has acted illegally in withholding highway funds. The New York lawyers said last week that they are prepared to go to the Supreme Court if necessary and that in any case the Supreme Court is the appropriate place to determine constitutional relationships between the White House and Congress.

important, the agency is considering rescheduling such drugs to put them in a more tightly regulated category because of their abuse potential. Amphetamines were, in fact, put into Schedule II—the most closely regulated category—a year ago, but some of the other related drugs were not rescheduled at that time.

Dr Simmons pointed out that rescheduling amphetamines immediately produced a dramatic decline in the number of prescriptions for them—the number of prescriptions dropped from 2 million a month to about 670,000—and he believes that when the other appetite-suppressants are rescheduled there will be a similar decrease in their use. The FDA's policy is therefore one of discouragement, but not of outright disapproval.

Asked by Senator Gaylord Nelson, chairman of the Monopolies Subcommittee, why the FDA decided to allow the drugs to remain on the market in spite of the triviality of their effect and their abuse potential, Dr Simmons replied that the problem of obesity in the United States is so great that doctors need every weapon at hand to fight it. Even though the effect of the drugs on average is trivial, they have been very beneficial in some cases, and should be kept as a last resort.

But other witnesses before the committee took issue with the FDA's position. Dr Jay Tepperman, professor of experimental medicine at the State University of New York, for example, pointed out that the efficacy trials lasted for only a few weeks, and no follow-up studies have so far been attempted with the patients involved. "It is astonishing that 'efficacy' in the case of amphetamine-like drugs is defined as the ability of the drug to induce a statistically significant excess weight loss over that achieved by diet and a placebo alone over a period of a few weeks", he said. "There is absolutely no evidence that the trivial additional weight loss attributable to this class of drugs has any effect whatever on the long-range fate of the obese patient." He concluded that use of these drugs in the treatment of obese patients "should be discouraged".

Similarly, Dr Meyer said that "in the case of amphetamines, the limited usefulness to some patients does not counterbalance the enormous societal cost brought about by the availability of 'pep pills'". But instead of banning appetite-suppressants outright, Dr Meyer suggested that the FDA should simply remove some products from the market, leaving only one or two for the treatment of such diseases as narcolepsy. "By no stretch of the imagination do we need the number or variety of preparations now in existence," he said. Such a policy would also stop the drug

companies from assaulting doctors with advertisements claiming that their products are highly effective. But the policy would, of course, be difficult to put into effect in a free market economy, and the FDA is therefore stuck with a policy of either discouragement or outright disapproval. At present it is opting for discouragement.

CLEAN AIR

Back to World War II

by our Washington Correspondent

THE Environmental Protection Agency's regional office in California has come up with a drastic plan for meeting the air quality standards prescribed by the Clean Air Act in Los Angeles and surrounding counties—petrol rationing. According to a study carried out by a technical committee appointed by the regional office, petrol consumption must be cut by 86 per cent between May and October if the area is to have a hope of meeting the air quality standards. Such a move, the office estimates, would eliminate nearly 200 tons of hydrocarbons emitted to the atmosphere each day, but even this would not be sufficient to meet the standards, and the remainder would

have to be removed by emission control devices on automobiles and industrial plants.

The Clean Air Act empowered the Administrator of the Environmental Protection Agency to set nation-wide ambient air quality standards, and the states were told to draw up their own plans for meeting them. Los Angeles and six other counties in Southern California said that they could not meet the standards, however, and so the EPA itself must draw up implementation plans. The rationing scheme is very preliminary and it has been sent to the EPA head office in Washington for approval. If approved by Mr William D. Ruckelshaus, Administrator of the EPA, rationing would start in 1975. Ruckelshaus has, in any case, been ordered by a court in Los Angeles to submit a final plan by January 15, 1973.

Clearly, as it now stands, the rationing plan would bring most of the Los Angeles area to a grinding halt, and even if it is approved by Ruckelshaus, it is unlikely that Congress would allow the plan to go ahead. Having seen that the standards prescribed by the Clean Air Act are too stringent in the Los Angeles area, Congress would probably be forced to make an exception—a move which could open up the floodgates for special pleading from other parts of the country. If Ruckelshaus does approve a modified version of the plan, at least he would put the ball firmly back into Congress's court.

HEALTH

Marston to Go

PRESIDENT NIXON'S reshuffle of the executive has made nearly a clean sweep of the federal government's top health officials. Latest casualty is Dr Robert Q. Marston, Director of the National Institutes of Health. Marston was informed last week that his pro forma resignation has been accepted, and that a new director for NIH will be appointed in the next few weeks. His dismissal follows the shifting of Mr Elliot Richardson from Secretary of Health, Education and Welfare to Secretary of Defense, and the resignations of Merlin K. Duval, Assistant Secretary for Health, and Vernon E. Wilson, Administrator of Health Services and Mental Health.

Marston's dismissal came as a surprise, because there had previously been no indication that Nixon wanted to replace him. He came to NIH in 1968, to replace Dr James Shannon, and during his four years in office, NIH's budget has increased from about \$1,300 million to an estimated \$2,100 million this year. Marston, who left for a meeting in Europe immediately after he received news of his dismissal, said he would probably remain at NIH in some capacity at least until next summer.

Short Notes

Ocean Currents

SCIENTIFIC agencies in France, the United States, Japan and the United Kingdom are cooperating on a project designed to obtain information on ocean circulation near the Antarctic continent. The plan is to place transmitters on several icebergs in the Antarctic and then to track their movements by Eole-1, a French satellite launched last year. Data resulting from the operation will be collected by the French National space research centre and distributed to the cooperating countries.

Next ACS President

DR BERNARD S. FRIEDMAN, a professor at the University of Chicago who has had a long career in the oil industry, has been chosen as president-elect of the American Chemical Society by mail ballot. Dr Friedman will take over as ACS President from Alan Nixon on January 1, 1974. As president-elect, he will join the ACS board in January for a three-year term. Dr Gardner W. Stacey and Dr David S. Breslow were also elected to serve three-year terms on the ACS board of directors.

NEWS AND VIEWS

Seafloor Spreading in the Philippine Sea

THE Western Pacific Ocean is ringed with marginal seas, island arcs like Japan and associated trenches. These systems lie between continent and ocean; the marginal seas occur to the landward of the island arcs and the trenches to the seaward. The island arcs have regular shapes and are nearly always concave towards the centres of the marginal seas.

The classical explanation of these marginal seas is that they were continental areas that had subsided. Seismic refraction studies have since shown them to have typical oceanic crust, so this explanation can now be ruled out. An alternative suggestion is that the marginal seas were formed by spreading: Karig has suggested that the basins were created by crustal extension; the island arc was cut in two longitudinally by extensional forces caused by hot diapirs rising from the Benioff Zone. As the arc and its associated trench were pushed oceanward, this split widened to open the inter-arc basin. Such a lineated intrusion process can be made compatible with rigid plate tectonics, but it requires a large number of small plates between the intrusion zone and the trench.

The heat flow, elevation and age of active mid-oceanic ridges are uniformly related. New plate material is produced at spreading ridges; the plate is cracked at the ridge crest, hot material rises, intrudes and moves away symmetrically from the crest. As it moves away it cools and contracts. If similar processes are going on in marginal seas then the same increase of heat flow with elevation should be observed. A high heat flow and a thin crust have been observed in many of the marginal basins in the western Pacific, although not in the Philippine Sea. McKenzie and Sclater (*J. Geophys. Res.*, **73**, 3173; 1968) have investigated the possibility of intrusion as a means of accounting for the high heat flow in the Japan Sea, but the spreading rates involved, 10 cm yr^{-1} , were considered too great to be likely.

On page 453 of this issue of *Nature*, Ben-Avraham, Segawa and Bowin report evidence of an extinct seafloor spreading centre in the west Philippine Basin. They have recorded bathymetry, magnetism and gravity along a profile over a ridge striking $\text{N}50^\circ\text{W}$. The profile is very like the one that would be expected of a mid-oceanic ridge. The observed profile of magnetic anomalies is nicely symmetric about its axis and is in quite good agreement with another profile made over the same part of the ridge in 1963. An airborne magnetic profile (Project Magnet) has also been made over the ridge, 5° to the west. Fewer anomalies were found there, but apparently they too are consistent with a symmetrical pattern. Ben-Avraham *et al.* attempted to construct a seafloor spreading model to explain the observed magnetic anomalies which can either be generated by a ridge striking $\text{N}10^\circ\text{W}$ at 15°N (the present latitude of the ridge) or striking $\text{N}50^\circ\text{W}$ (the present strike of ridge) at latitude 8°N . They favour the second possibility. Furthermore, they were unable to correlate the symmetric profile with any simulated anomalies computed from the Cretaceous time scale, and so it would seem that the anomalies were probably formed in the Mesozoic.

A comparison of the ridge elevation with the depth/age plots of Sclater *et al.* (*J. Geophys. Res.*, **76**, 7888; 1971) gives a minimum age of 58 million years for the ridge crest. If marginal seas turn out to be no different from any other seas, then this comparison should be valid, provided that rates of cooling and sinking have not been affected by the other complicated processes involved in the Tertiary formation of Kynsha Ridge, Parece-Vela Basin and the Mariana Island Arc system.

It would be very pleasant if, in fact, there is Mesozoic seafloor in the west Philippine Sea. If deep sea drilling can date these symmetric and well-defined anomalies, then they should provide a key to a good Mesozoic time scale, which could then be related to other parts of the Pacific. More work must be done along the ridge to check that the anomalies are continuous, and thus to ensure that the ridge was a spreading centre.

In the past few years spreading ridges have been postulated, on magnetic evidence, in the Scotia Sea and also in the Lau Basin. As these ridges are spreading parallel to the island arcs they can account for the formation of the marginal seas. Magnetic lineations have, however, been sought, but not found, in several of the marginal seas. One would not expect to find magnetic lineations in a marginal sea formed by diapirs rising from the Benioff Zone behind the island arc. Certainly this model is therefore not valid for the west Philippine Sea.

In next Monday's issue of *Nature Physical Science* (December 25) Uyeda and Ben-Avraham suggest an ingenious speculation on the origin of the Philippine Sea in the light of this discovery of the Philippine Ridge as a spreading centre. The Philippine Sea is separated into dissimilar western and eastern halves by the north-south Kyushu-Palau ridge. The western basin has thicker sediments and northwest-southeast features, including the Philippine Ridge. The eastern basin has north-south features, and on geological grounds, including two JOIDES holes in the Parece-Vela Basin, it would seem that neither the eastern Philippine Sea nor the island arcs existed in pre-Eocene times (60 million years ago). Their suggested method of formation combines the seafloor spreading of the Philippine Ridge in the Mesozoic and Karig's island arc splitting in the Tertiary to account for this.

A hundred million years ago, when the Philippine Ridge was spreading, they place it further to the south than at present and connected to the Kula-Pacific ridge by a long transform fault. To the north was the Kula Plate, moving north-northwest and being subducted by the Ryukyu-Japan-Kurile trench; it is assumed to have submerged under the Japanese Islands about 80 to 90 million years ago. From the figures it seems that the Philippine Ridge was striking $\text{S}75^\circ\text{W}$ throughout this period, which does not seem to fit in with the conclusions of Ben-Avraham *et al.*, that the ridge could only generate the observed magnetic anomalies if it were striking $\text{N}50^\circ\text{W}$ at 8°N .

Uyeda and Ben-Avraham then make use of the change of motion of the Pacific Plate about 40 million years ago from north-northwest to west-northwest, as predicted by

the controversial "hot spots" theory, and suggest that the Philippine Ridge (by then extinct) may not have changed its motion to the same extent. According to the authors this would produce the interesting situation in which a transform fault becomes a subduction zone. It is also interesting to note that this zone is continued to the south beyond the Philippine Ridge. These postulated movements of the Pacific Plate must be examined to see how they relate to the magnetic lineations in the West Pacific. During the Eocene (40 to 60 million years ago) Uyeda and Ben-Avraham suggest that the transform fault became an island arc, which, 40 million years ago, split into two by the Karig process to form the Parece-Vela Basin. From then on Karig's crustal extension model explains the formation of the Parece-Vela Basin and Mariana Island Arc system.—C. M. R. F.

Steady State Obituary ?

FOR more than fifteen years after its invention in 1948, the steady state cosmology of Bondi, Gold and Hoyle exercised a strong hold on the imaginations of British astronomers, and probably an even stronger hold on the imaginations of those members of the wider population with a philosophical turn of mind. Those secularists who were unsatisfied by the discomfiture of established religion by Copernicus, Galileo and Darwin presumably rejoiced at the abolition of the Creation altogether. The absence of an end of the world, a matter inconveniently undecided by conventional big-bang cosmology, was obviously of benefit to all. The Universe was to continue for ever to present the same face to us, the recession of the galaxies compensated by the formation of new ones from continuously created matter. The fact that at that time the age of the Universe according to the big-bang picture seemed to be very much shorter than the age of the Galaxy provided additional support for those for whom philosophical arguments are insufficient.

Outside Britain the theory had fewer converts, although whether this was due to conservatism, or to inconsistency with the Book of Genesis or Dialectical Materialism, is unclear. Even in Britain the theory had its enemies. One radio observatory was rumoured to have decided to commit its entire scientific effort to the destruction of the theory. Certainly it was radio source counts that provided the first stumbling block. As the Universe is to be the same at all places and all times, one has only to study the properties of a sample of it in order to be able to predict the behaviour of the rest. In a recent issue of *Nature* (240, 399; 1972) M. Schmidt uses as his sample a set of twenty-six completely identified sources brighter than 30 flux units at a frequency of 178 MHz to predict the number of sources expected in the steady state theory at fainter flux levels. The number actually observed is greater than the steady state prediction by a large factor which depends on how far away the quasars are presumed to be.

Inconsistency of the steady state cosmology with radio source counts was claimed by Ryle and Clarke back in 1961, before quasars had been discovered. Hoyle and Narlikar responded with an extremely ingenious modification of the steady state idea (*Mon. Not. Roy. Astron. Soc.*, 123, 133; 1961). The continuous creation required

by the theory was to occur in the form of massive (about 10^5 galaxies) discrete primary condensations at irregular intervals, instead of taking place at a uniform rate at all places and all times. Within one of these condensations an expanding system of objects all of the same age would be seen. If the probability of a galaxy being a radio source depends on age, an apparent evolutionary effect would be seen within the condensation. The Universe is in a steady state on average, but coherent temporal fluctuations occur over extended regions.

Although this modified steady state theory has been for the most part ignored by supporters of the big-bang, it is probably fair to say that the modification was never fully developed by its proponents. Of course, the severe simplicity of the original idea has been lost. Critics argue that it is necessary for one to be in rather a special place, near the centre of one of these condensations, in order to avoid strong anisotropy in the distribution of radio sources. The distribution of galaxies brighter than 16 mag is, however, not especially isotropic, and it would be surprising if radio sources do not ultimately reflect this anisotropy.

A more serious difficulty for the steady state theory was the discovery of microwave background radiation by Penzias and Wilson in 1965. Apart from some as yet unresolved anomalies in the millimetre waveband, this radiation has the spectrum of a 2.7 K black body and is exceedingly isotropic, consistent with being a relic of the fireball phase of the big-bang. *Pace* the radio astronomers, it is this microwave background radiation which has turned most steady state supporters into big-bang bigots.

By 1969 Hoyle had shifted to much weaker ground. In his Bakerian lecture (*Proc. Roy. Soc. A*, 308, 1; 1969) he suggested that the source counts at faint flux levels fit the steady state predictions well: all that is wrong is a deficiency of four or five sources per steradian at high flux levels. This figure was based on back-of-envelope considerations of the unidentified sources in the 3C survey. On the basis of a more detailed calculation, Maarten Schmidt argues in his report that his sample of twenty-six sources would in fact have to be deficient by a factor of five, far too much for a statistical fluctuation.

Whether the redshifts of quasars are entirely cosmological is still the subject of hot debate, as may be seen from the many articles on the subject in *Nature* during the past year. Schmidt concludes that "whatever is the interpretation of the quasar redshifts, the observed radio source counts, identifications and redshifts are incompatible with the steady state cosmology". This is true provided the quasars in his sample are typical representatives of a population of objects spread uniformly through space. But if, first, all quasars belong to a local irregularity; second, most unidentified sources are quasars; third, many identifications of radio sources with galaxies fainter than 18 mag are mistaken (otherwise the distribution of radio galaxies alone is inconsistent with the steady state); and, fourth, the microwave background is not cosmic black body radiation but is, for example, due to starlight reradiated by dust (Hoyle drew attention in his Bakerian lecture to the similarity of the energy densities involved), then even the strict steady state cosmology could perhaps be saved.

The strong arguments that can be mounted against all four of these conditions seem, however, entirely convincing.—M. R.-R.

Trace Amounts of Radioactivity in Materials

THE abundance and extent of natural radioactivity are such that human life is passed, and always has been passed, to the accompaniment of disintegrating nuclei and against a background of emitted radiation. Principal sources of natural radiation are uranium, thorium (and their daughters) and potassium in rocks and soil, together with the cosmic radiation. In modern times this natural burden of radiation has been increased by the introduction of artificial radioisotopes in medicine and industry and by the testing of nuclear weapons, as well as by the medical use of radiation for diagnosis and treatment.

The level of dose to human beings caused by this universal exposure to nuclear radiation and radioactivity is regularly considered by the United Nations Scientific Committee on Atomic Radiation. The biological significance of these dose levels is appraised by another careful and responsible body, the International Commission on Radiological Protection. By these means the risk to human health and life is controlled by regulation and recommendation.

Meanwhile radioactivity can be created, broadcast and adventitiously accumulated at levels well below those of official notice and concern. The sensitivity of modern techniques of detection can be orders of magnitude below levels of significance to humans and it is thus at the bottom of the scale of radioactivity that the report on radioactive silver by Lindner *et al.* (page 463 of this issue of *Nature*) has relevance.

The appearance of yet more radioactivity embodied in material that might be used in apparatus for counting at a low level must cause dismay. The problems associated with trace amounts of natural radioactivity in counter materials have been long recognized. Radiation shields of lead less than a century old could contain detectable amounts of ^{210}Pb and photomultipliers used in scintillation counters should have quartz and not glass windows, because of the possible presence of ^{40}K . Even photographic emulsions are sensitive to the content of uranium in the glass backing.

The list of examples of interference from artificial radioisotopes must be restricted to a few that come readily to mind. Increasing use of ^{60}Co as a tracer in the manufacturing processes causes modern steel to be contaminated. Again, lead is implicated because of ^{125}Sb , possibly from radioactive fallout, in antimonial lead (see *Nature*, **213**, 796; 1967). ^{85}Kr , a fission product from atomic fuel processing, is an actual or potential contaminant of krypton-filled gas counters. In fact, most materials used in the construction and shielding of counters are suspect (Watt and Ramsden, *High Sensitivity Counting Techniques*, Pergamon, London; 1964) as are chemical reagents. No knowledgeable practitioner would proceed before making a thorough investigation of samples of the materials to be used.

What are the solutions? The appointment of another international committee or commission is obviously unnecessary and undesirable. It will simply have to be accepted as a fact of life that the supply of materials free from radioactivity for low level counting measurements has become

more difficult and expensive because of the need to test and select.—From a Correspondent.

SIMIAN VIRUS 40

Transcriptional Control

from our Cell Biology Correspondent

AFTER sailing in the doldrums for many a year virologists, intent on elucidating the way in which the transcription of SV40 DNA is regulated in permissive cells supporting the replication of this virus and non-permissive cells transformed by it, now suddenly find themselves racing each other before a gale of new observations. This sudden change in climate stems from two innovations. First, as Khoury *et al.* and Sambrook *et al.* reported during the summer, it is possible to use the RNA transcribed asymmetrically *in vitro* by *Escherichia coli* RNA polymerase from double-stranded, covalently circular SV40 DNA to effect the separation of the two strands of SV40 DNA. Second, as Danna and Nathans discovered last year, a restriction enzyme from *Haemophilus influenzae* cuts SV40 DNA at specific sites to yield eleven specific fragments of the SV40 genome and the two strands of each fragment can be separated with the *in vitro* SV40 RNA.

A Foetal Myosin in the Rabbit

HUSZAR, in next Wednesday's *Nature New Biology* (December 27), gives evidence that there is a rabbit foetal skeletal muscle myosin which differs in its sequence from that of the adult form. It is known that the myosin of adult rabbits contains methylhistidine, and also mono and trimethyllysine residues, all in the globular heads of the protein. The fully methylated protein seems to contain one methylhistidine and two trimethyllysines per head.

Whereas trimethyllysines occur in the myosins of all muscles so far examined, the methylhistidine is not found either in cardiac or slow muscle myosin, or in the skeletal myosin of newborn rabbits. The methylhistidine appears progressively during the first month of life, and Huszar has accordingly isolated the partially methylated histidine-containing peptide from the myosin of three-week-old rabbits. The methylated and unmethylated forms of the peptide elute together from columns.

Comparison of the amino-acid composition with that of the corresponding fully methylated adult myosin peptide shows that the contents of glycine and

valine are lower, and those of isoleucine and alanine higher, in the maturing form. Sequence analysis shows that the latter is a mixture of two peptides, one of them identical with the adult counterpart. Thus there are in this thirteen-residue peptide two conservative replacements, both corresponding to single-base mutations. In the corresponding peptide from adult rabbit cardiac myosin there are four differences from that of skeletal, one of which is in the same position as one of the two replacements in foetal skeletal myosin. By contrast, trimethyllysine-containing peptides isolated from adult and foetal skeletal myosins show no differences.

Huszar suggests that, in view of the conservative nature of the substitutions in the methylhistidine region, the different degrees of methylation are caused not by the failure of the methylating enzyme to recognize the substrate, but by its absence in the foetal tissue. There are evidently at least three genes for myosin heavy chains. The degree of methylation seems to be correlated with the speed of muscle contraction.

It has now been possible to confirm that about 30 per cent of one strand of SV40 DNA (that which is transcribed *in vitro* by *E. coli* polymerase) is transcribed during lytic infections before viral DNA replication occurs to yield stable "early" SV40 RNA; whereas, after viral DNA replication begins, in addition to these early RNA sequences about 60 per cent of the complementary DNA strand is transcribed into stable late SV40 RNAs. These findings imply that a strand-switching event occurs during the regulated transcription of the SV40 genome as the virus replicates in permissive cells. According to Patch *et al.* (*Proc. US Nat. Acad. Sci.*, **69**, 3375; 1972) the crucial segment of the SV40 genome that contains this transcriptional control region may be accessible to investigation in a non-defective human adenovirus 2-SV40 hybrid virus.

Patch, Lewis and Levine have been working with an Ad2-SV40 hybrid virus which is called Ad2⁺ ND₁. This virus has a genome which contains some 10-18 per cent of the SV40 genome (estimates of the amount of SV40 DNA vary with the techniques used to measure it) covalently linked to a human adenovirus 2 genome carrying a 5 per cent deletion. When this hybrid virus infects monkey cells and replicates some SV40 early RNA is transcribed and SV40-specific U antigen is induced as it is early during infections of monkey cells with intact SV40. These observations indicate that at least some of the SV40 DNA that is transcribed early during replication is present in the segment of the SV40 genome in the Ad2⁺ ND₁ hybrid. That this is indeed the case has been confirmed by Patch *et al.* who first devised a method for separating the two strands of Ad2⁺ ND₁ DNA and then showed that SV40 specific RNA transcribed *in vitro* by *E. coli* RNA polymerase hybridizes to one of the two strands of hybrid virus DNA.

But are any of the late SV40 DNA sequences also present in the small segment of SV40 DNA in the Ad2⁺ ND₁ genome? To answer this question Patch *et al.* challenged the separated strands of hybrid virus DNA with SV40-specific RNA extracted at late times from cells infected with SV40 and supporting its replication. This RNA contains both early and late sequences and some of it hybridizes to one strand of Ad2⁺ ND₁ DNA while some of it hybridizes to the other Ad2⁺ ND₁ DNA strand. In other words, the SV40 DNA in the hybrid virus contains sequences complementary to both early and late SV40 RNAs. This must mean that the 10-18 per cent of the SV40 genome in the hybrid virus must include the SV40 early/late transcriptional control region. Clearly this hybrid virus and the four others belonging to

the same family are proving to be more than virologist's curiosities; they are tools for probing further the nature of the signals which initiate and/or terminate transcription of SV40 DNA.

Another useful tool for analyses of the structure and function of SV40 DNA is described in a crop of reports in the same issue of the *Proceedings* (*ibid.*, 3215, 3365, 3370, 3448). All these reports are concerned with one property or another of the R₁ restriction endonuclease that can be isolated from *E. coli* carrying the drug resistance transfer factor RTF₁. This enzyme converts circular duplex SV40 DNA into linear unit length duplex molecules by cleaving both DNA strands at a unique site which can be mapped with respect to other physical markers and by partial denaturation mapping. Obviously the last has not been heard of this enzyme or the non-defective adenovirus 2-SV40 hybrid viruses which could, for example, be teamed together in some interesting experiments.

CONTRACEPTION

Switching Off the Male

by our Special Correspondent

"THE only world for global survival is the contraceptive world." Male contraception should be culturally acceptable and completely effective; it should be non-toxic, have specific and reversible action and have no effects upon male libido and complete fulfilment of sexual union. These were the conclusions of a workshop on "Contraception: The Masculine Gender", organized by Schering AG in Berlin, from November 29 to December 2. The organizers had gathered together a diverse group of scientists with a wide

range of interests in an attempt to answer the question "Why bother with the male?" The fact of the matter is that contraception, with the advent of the oral steroid pills, has become the responsibility of the woman; the more difficult problem of male contraception has been largely ignored.

The testis and epididymis are the chief target sites for contraceptive action. Maleness begins with the differentiation of the testes early in foetal life and must impose over femaleness. Dr A. Jost (University of Paris) discussed this development and stressed the importance of the Sertoli cells, which appear quite suddenly in the testis, swell and within a few hours completely encompass the germ cells. The control of this process is not yet known but, together with the interstitial cells, the Sertoli cells probably "fight" the female problem.

An unusual feature of the Sertoli cell membrane was presented by Drs D. Fawcett and B. Gilula (Harvard Medical School). In order for any agent to affect spermatogenesis it must penetrate the seminiferous tubules. The epithelium, which at some point must allow upward movement of new spermatocytes, has two compartments—a basal region to which materials have direct access and an adluminal region with developing cells to which substances are denied access by the Sertoli cell junctions. These junctional complexes are unlike any other epithelium and at some places the apposing cell membranes are very close and appear to fuse to form a blood/testis barrier. Beautiful freeze-etched pictures of these areas were presented from experimental rodents. This technique cleaves the cell membrane to reveal the outwardly directed inner membrane (A face) and the inwardly directed outer membrane (B face). Cleavage at

Locating Yeast Ribosomal RNA Cistrons

IN next Wednesday's *Nature New Biology* (December 27), Finkelstein, Blamir and Marmur report the development of a technique for analysing intact chromosomal DNA molecules in yeast. After labelling, intact DNA molecules are released on the surface of sucrose gradients by detergent lysis of spheroplasts. By these means it is estimated that the linear duplex molecular weight of yeast DNAs lies between 400 and 700 × 10⁶.

It is known that the size of yeast rDNA is at the upper limit of this molecular weight range and that in most organisms rRNA cistrons are clustered so that it is thus possible that all the rDNA is located on one chromosome.

DNA-rRNA hybridization experiments showed, however, that it is more probable that all the rDNA is accommodated on two chromosomes, one

of which contains mostly rDNA. A likely candidate for the latter chromosome is yeast chromosome 1 because it contains only one known genetic marker. Comparison of labelled DNA in a monosomic (2n-1) strain of yeast, haploid only for chromosome 1, with DNA of its isogenic diploid showed that the molecular weight of this chromosome is of the right magnitude. Control hybridization experiments showed that none of the other species of DNA in this size class hybridized to rRNA. Ribosomal DNA is thus located on chromosome 1.

Other experiments showed that the rDNA content of chromosome 1 accounts for 70 per cent of the total rRNA genes and there is evidence that the remaining 30 per cent of the rRNA genes are also clustered on one or more chromosomes.

points of fusion of Sertoli cells showed particles in linear arrays on the *A* face with corresponding pits on the *B* face. This unique, occluding junction would be an effective barrier, protective against substances in circulation. This barrier, suggested Dr Fawcett, could perhaps be opened up to allow the penetration of a contraceptive substance.

Dr P. L. Pearson (State University of Leiden) discussed the manipulation of the action of the *Y* chromosome genes and the production of an abnormal gamete as a possible method of contraception. Of the three phases of DNA synthesis (*S* phases), those occurring at preleptene and zygotene in the meiotic cycle have normal repair replication patterns and any interruption of synthesis here would be unspecific for any meiotic cell. At the third *S* phase, at pachytene, there is abnormal repair, however, and if a particular endonuclease is featured here, then a block at this stage would be specific for spermatogenesis. Although most of this research has been carried out in plants (*Lilium* sp.), there is evidence that similar repair mechanisms occur in mammalian cells.

Spermatogenesis was also the target of Dr D. Lacy (St Bartholomew's Medical School, London), who discussed the possibility of reducing the testicular weight (and thus spermatogenesis) while maintaining or stimulating the seminal vesicles using testosterone or a synthetic androgen. This differential response could perhaps be achieved by using a centrally acting oestrogen to prevent local production of steroids, followed by androgen administration to stimulate the seminal vesicles to take up testosterone. Work in his laboratory with rats suggests that this differential response might also be achieved in man.

Dr J. M. Bedford (Cornell University Medical College) suggested contraception by a chemical change mediated through the sperm cell surface which would affect its ability to fertilize the ovum. His study on the maturation changes as sperm pass through the epididymis concentrated chiefly on four aspects—the acrosome, the surface membrane, the nucleus and motility. Acrosome morphology was unchanged, but he found significant changes in the cell surface, the state of which is critical for the ability to fertilize. The nucleus also showed changes in the degree of covalent cross-linking. The available sulphhydryl groups provided by the cysteine of the nuclear protein become cross-linked and confer greater structural rigidity on the mature sperm. The capacity for progressive movement rather than weak tail thrashing is accompanied by changes in the quality of the tail fibres during maturation; modifications to render sperm immobile would be difficult, but Dr Bedford suggested that they may be

worth considering. Changes in nuclear development, however, are of functional importance in embryonic development and it was argued by Dr R. A. Beatty (University of Edinburgh) that the non-nuclear change affecting, for example, the accessory glands was far preferable to damage of genetic information and possible mutagenicity.

There was a considerable body of opinion that the epididymis is the site for contraceptive action. Dr D. Hamilton (Harvard Medical School) discussed its physiology as related to anatomical structure and showed that interference with any constituent of the lumen resulted in impairment of the functional integrity of the epithelium.

Dr G. Waites (University of Reading) described the passage of substances from the blood into the testicular lymph and fluid. All pass freely into the lymph but can be divided into three groups depending on their rate of entry into the testicular fluid, which does not depend upon molecular weight. A steroid could therefore be altered to prevent its diffusion but still act systematically as an androgen. Dr Waites suggested that this could be a useful screening technique, and the epididymal sterilant could be designed to act upon the rete testis. He warned, however, that, because of the similarity of the testicular fluid and brain fluids, the "ideal" fluid might also enter the blood/brain barrier; cerebrospinal fluid should therefore be

included in the screen.

The technique described by Dr Waites for continually collecting large quantities of fluid for a long time without contact with the accessory organs greatly aided the study of metabolism and survival of sperm in the epididymis by Dr I. G. White (University of Sydney). He has found that testicular sperm metabolizes and incorporates glucose in the form of inositol, suggesting that this may be a reserve form of carbohydrate for sperm passage through the tract. The high concentration of phospholipid in testicular sperm becomes reduced as they pass along the epididymis, but the lipid does not appear in the plasma and Dr White suggested there may well be a sodium and potassium pump to maintain cation gradients. This, he felt, could perhaps be exploited in the development of a contraceptive.

The characterization of an acrosomal enzyme, acrosin, was described by Dr H. Fritz (University of Munich). Active acrosin is required for penetration; artificial inactivation either by interfering with its natural inhibitor or by permanently inactivating the enzyme could lead to new contraceptive ideas.

The place of the routine screen for a male contraceptive was discussed by Dr M. M. Ketchel (Oak Ridge Population Research Institute), who pointed out that it should be possible to develop compounds without side effects by either utilizing enzymes affecting the

Fluctuating Numbers of Ribosomal Genes

It has been assumed that, with certain notable exceptions, the content of ribosomal RNA cistrons in each cell is a fixed number for any given genotype. Howell now reports in next Wednesday's *Nature New Biology* (December 27) that, in *Chlamydomonas reinhardtii*, fluctuations in the genome content of rDNA occur during a single cell cycle.

When labelled rRNA was extracted from *Chlamydomonas* cells and run on an MAK column, two fractions were obtained—an 18S species and a mixed fraction containing 18S and 25S rRNA. It was then calculated, from DNA-RNA hybridization experiments with these fractions, that the number of rRNA cistrons per cell in continuously growing cultures is 400 for 18S rRNA and 250 for 25S rRNA. Both 18S and 25S rRNA hybridize to a DNA fraction of unique buoyant density.

In synchronized cells, however, the percentage of DNA which hybridizes at saturation to rRNA varies during the cell cycle. For both 18S and 25S species the amount of rDNA per cell rises sharply after cell division, then increases more gradually until the next division when the content of rDNA per cell falls abruptly. The difference in

the values of the highest and lowest number of rDNA cistrons per cell during the cycle represents more than a three-fold change in the cellular content of both species of rDNA.

Howell correlates the pattern of rDNA degradation and synthesis with breakdown and reformation of the nucleolus during the cell cycle. He proposes a model relating rDNA metabolism with the nucleolar cycle. A limited number of rRNA cistrons, located at the nucleolar organizer, are considered to be an integral part of the chromosome. Additional non-chromosomal copies of the rRNA cistrons exist as part of the nucleolar structure. They are degraded during nucleolar breakdown and are not passed on to daughter cells. Following division and during nucleolar reformation additional non-chromosomal copies of rDNA are resynthesized.

According to the model the cellular content of rDNA at any point in the cell cycle will be the product of synthetic and degradative control of non-chromosomal rDNA and, in cells which have "persistent" nucleoli during mitosis, there is no degradation and re-synthesis of nucleolar rDNA.

reproductive tract but ineffective elsewhere or developing specific enzyme inhibitors. He felt that screening great numbers of compounds should not be discouraged, and the non-commercial aspect of this would prevent dismissal of compounds not commercially viable. Dr Ketchel has so far included 257 compounds in his screening programme; fifteen di-ethyl amino-ethanol and related compounds gave anti-fertility activity. The mode of action of these compounds is unknown but they do act in the female. Dr Ketchel pointed out, however, that there is an important place for a screening programme in the search for a male contraceptive. In discussion the need for a central registration of screening results was stressed.

Dr R. Ericsson (Schering AG) suggested that the criteria for a successful antispermatic drug were oral or intramuscular administration, rapid depletion of sperm, lengthy azoospermia, return of fertility on withdrawal, and normal libido; it must be non-teratogenic, have selective pharmacology, be free from serious side effects and have a good therapeutic index. In discussion it was also felt that screening should include the cerebrospinal fluid, because of the similarity of the blood/brain, blood/testis barrier, and a test for psychological toxicity. Dr H. Ginsburg (Chelsea College, London) suggested that the partnership should be regarded as the drug-taking unit and not the individual.

The workshop also discussed the psychological aspects of male contraception, the use and abuse of available methods and the utilization of the "on/off" mechanism in seasonal mammals to "switch off" the male.

It is clear that male contraception still lies in the future; more knowledge is required of all aspects of male reproduction and, in order to achieve this, all possible approaches must be studied and coordinated at further meetings of this kind. It was, however, generally felt that when "the era of the male" arrives it will only be a further alternative to present methods and will not supersede them.

GRASSLAND MANAGEMENT

Role of Nutrients

from our Plant Ecology Correspondent

BIOLOGICAL succession is a subject which has attracted the attention of ecologists since the days of Clements. The sequential process whereby plant and animal communities change and develop until the attainment of a stable, climax state has been described in detail from a variety of habitat types, especially sand dunes and glacial detritus. Two aspects of succession which have not received

adequate documentation are the role of nutrient accretion in succession and the relation between this accretion and floristic diversity.

B. H. Green now draws together in an interesting article (*Biol. Conserv.*, **4**, 378; 1972) the fragments of information which exist on this subject and applies to them his experience of habitat management. The unified and convincing thesis which results associates the high floristic diversity of communities from early successional stages (for example, chalk grassland) with soil infertility.

For decades many ecological problems associated with plant succession on chalk grassland have been neglected because of the British ecologist's obsession with the question of adaptation to life in calcium carbonate rich substrates (the "calcicole—calcifuge" problem). Often a confusion has arisen in which high pH has been considered equivalent to nutrient richness, but in fact the reverse is true. Chalk soils are rich in calcium ions, but poor in nitrogen, phosphorus and potassium. Moreover, past management systems of chalk grasslands have tended to contribute to their nutrient poverty.

The chalk grasslands of south-east England have developed as a result of centuries of a distinctive type of man-

agement practice. Sheep have been herded on the Downs during the day and kept in folds in the valleys during the night. This has resulted in a constant migration of nutrient elements from the hills into the valleys. Furthermore, there has been a regular cropping of stock for wool and mutton and this may also have represented a significant nutrient depletion especially on a substrate where nitrogen, phosphorus and potassium are in short supply. Some results suggest that losses to the ecosystem from this cropping exceeded the gains from rainfall, at least for nitrogen and phosphorus. Green believes that this management system maintained the low nutrient status of these grasslands for many centuries. He also associates this nutrient deficiency with high floristic diversity. Green quotes instances in which invasion by nutrient-demanding vegetation, which is often composed of bulky, vigorous species, leads to an increase in the rate of nutrient accretion within the ecosystem. This increase is associated with a reduction in floristic diversity.

The implications of these observations from the point of view of management and conservation are considerable. If the aim of such management is the maintenance of diversity, then all efforts should be made to retain or recover soil

More About Solar Neutrinos

FOWLER'S recent speculation that the absence of detectable neutrinos from the Sun might be explained if the Sun is in an intermediate phase, so that the nuclear reactions at present going on in its interior are not typical of a steady state situation (*Nature*, **238**, 24; 1972), has led to a flurry of activity among those astronomers who possess stellar evolution computer programs. Earlier this month, Dilke and Gough presented very detailed calculations of the effect of mixing on the Sun's core; this could lead to a temporary depression in the flux of solar neutrinos, associated with a fall in the Sun's temperature and hence an ice age on Earth (*Nature*, **240**, 262; 1972; see also **240**, 251; 1972). In next Monday's *Nature Physical Science* (December 25) two contributions, from Rood and from Ezer and Cameron, tackle the problem from slightly different points of view.

The differences between these investigations emphasize that the conclusions rest very sensitively on the details of the mixing. At one extreme, Dilke and Gough simulated a repeated mixing of the Sun by evolving a model for a simulated 4.45×10^9 yr with the core continuously mixed, then allowing "normal", unmixed evolution for 2.5×10^8 yr before suddenly mixing $0.25 M_{\odot}$ at the centre; this produced a flux of

around 1 s.n.u., in line with the latest observed upper limits. But Rood, mixing $0.25 M_{\odot}$ of a previously unmixed model, which might naively be expected to produce more drastic effects, finds a minimum of 7.3 s.n.u.

Certainly, repeated mixing during the life of the Sun seems a happier supposition than the one that we are living just after a unique event. Ezer and Cameron lean further in this direction, and mix a solar model twice, taking the second "event" as more typical of an intermittently mixed Sun. Mixing $0.56 M_{\odot}$, they find 0.8 s.n.u. after the first mix and 0.5 s.n.u. after the second, well below the limits placed at present by observation. But again, when Rood mixes $0.5 M_{\odot}$ of his model once, he finds a flux of 1.6 s.n.u., which emphasizes only too clearly just how sensitive to the model these studies are. These three contributions have sketched the outline of what might happen when models of the Sun are mixed in different ways. What is needed now is not more calculations of individual models considering one mixing mass fraction at a time, but more detailed (and necessarily more tedious) studies from which some consistent story might emerge now that it is known that some forms of mixing can reduce the solar neutrino flux adequately.

infertility. This may involve the cropping of nutrient rich vegetation and its removal from the system. The nutrient drain thus imposed should ideally equal the nutrient input from rainfall, rock weathering and biological fixation. Much more information about the nutrient cycling processes within ecosystems such as chalk grassland is required if their management is to be scientifically based.

NEUTRINOS

Double Beta Decay?

from a Correspondent

THE neutrinos which are believed to be emitted in thermonuclear reactions in the Sun's interior may complicate study of the phenomenon of double beta decay, according to Bozoki and Lande of the University of Pennsylvania (*Nuovo Cim.*, **12B**, 65; 1972).

Although beta decay usually involves the conversion of one neutron in a nucleus into a proton, an electron and an antineutrino, it may be possible for two neutrons to decay simultaneously if energy would be released by such a decay. Although the decay is highly improbable and would not be observable in a laboratory experiment, attempts have been made for some time to detect it through a study of old rocks (*Fortsch. Phys.*, **18**, 449; 1970).

The technique involves searching for traces of the isotope with $N-2$ neutrons and $Z+2$ protons in a rock containing the isotope with N neutrons and Z protons. The technique is particularly applicable if decay to the intermediate isotope with $N-1$ neutrons and $Z+1$ protons is energetically impossible, so that only double beta decay could have produced the trace of isotope ($N-2$, $Z+2$). Studies of this kind have led to double beta decay lifetimes between 10^{20} and 10^{21} years for the isotopes ^{130}Te and ^{82}Se and have shown that the lifetime of ^{128}Te has a lower limit of 10^{23} years.

In their recent article Bozoki and Lande suggest that neutrinos from the Sun can make it appear that double beta decay has occurred when in fact it has not. A neutrino can be absorbed by the (N, Z) isotope and can convert it into the ($N-1$, $Z+1$) isotope with the emission of an electron. As this isotope is unstable it subsequently decays into the isotope ($N-2$, $Z+2$) and the net result is that double beta decay has apparently occurred.

Bozoki and Lande have used the presently predicted neutrino flux from the Sun to calculate the simulated double beta decay lifetime of several isotopes. There is, of course, serious argument at present about this neutrino flux (see *Nature*, **240**, 262; 1972). The smallest lifetime that they have found is $\sim 10^{27}$

years so that it is unlikely that solar neutrinos have affected the apparent lifetime of ^{130}Te and ^{82}Se . These simulated lifetimes are not measurable with present experimental techniques, although they may be achieved eventually.

It is also possible to simulate double positron decay by the absorption of antineutrinos emitted by decay of radioactive elements in the Earth's core. Bozoki and Lande have used an estimate of the total amount of radioactive material in the Earth, and the consequent antineutrino flux, to calculate the simulated double positron decay lifetime of several isotopes. In this case the smallest predicted lifetime is of order 10^{28} years.

The great size of these simulated lifetimes suggests that simulated beta decay has not in fact been very important, at

least for isotopes for which accurate measurement is likely; but Bozoki and Lande furthermore point out that, although it is believed that the Sun must have been the chief source of cosmic neutrinos for most of the Earth's history, a very near supernova might have flooded the Earth with a very intense flux of neutrinos for a very short time. They suggest that evidence for such an occurrence might be found by studying apparent double beta decay lifetimes in rocks of different ages.

In fact an intense enough flux to give measurable results seems very unlikely and neutrino archaeology will probably be a cunning idea which cannot be used in practice. Recent experience in astronomy has, however, suggested that apparently implausible ideas should not be rejected too readily.

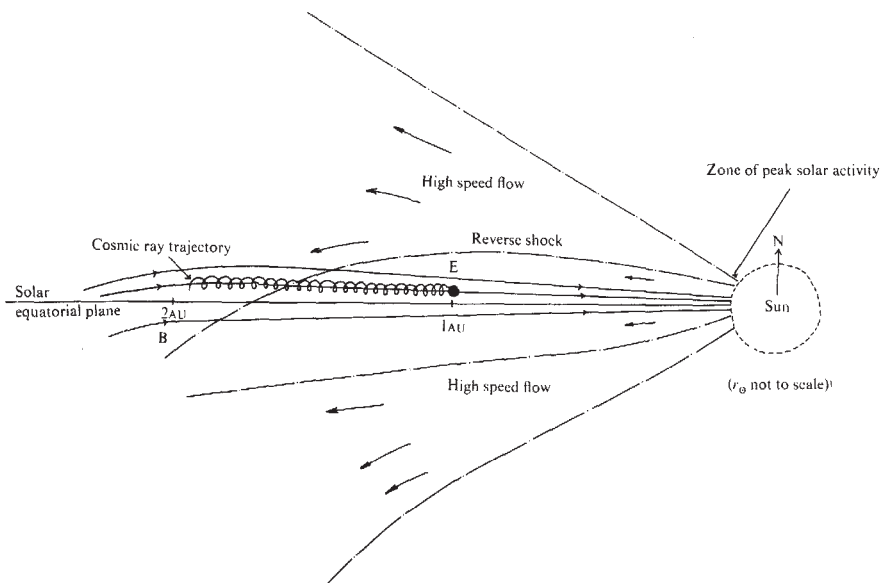
What Controls Cosmic Ray Modulation?

ALTHOUGH nobody doubts that the solar wind is instrumental in causing the flux of cosmic rays arriving at the Earth to alter slowly with time, there are some quite serious uncertainties about the exact manner in which the modulation occurs. Hedgecock, Quenby and Webb draw attention to some of the problems in next Monday's *Nature Physical Science* (December 25) and come up with a possible solution.

In essence they have to explain why measurements of the neutron flux at the Earth's surface, which reflects the flux of charged cosmic rays above the Earth's atmosphere, do not correlate with parameters like the velocity (V) and plasma density (ρ) of the solar wind as measured by satellites such as Vela 3, 4 and 5 and HEOS 1 and 2. Between the beginning of 1970 and the beginning of 1972, for example, the number of neutrons detected at one station went

up by 9 per cent, but this increase was not accompanied by the expected changes in the wind parameters.

Hedgecock and his colleagues suggest that phenomena happening well away from the ecliptic plane may be the real controlling factors; their model is shown in the diagram. The zones of enhanced solar activity in the northern and southern solar hemispheres could possibly cause the kind of distorted arrangement shown, for the northern centre of activity is known to be dominant. Cosmic rays spiralling in towards the Earth as in the diagram will thus tend to spend much of their time in the region of high speed flow to the north, and will consequently be affected more by the state of affairs there. Hedgecock *et al.* say that "clearly an off-ecliptic spacecraft is required to investigate these ideas thoroughly".



A possible plasma flow configuration in a solar meridian plane.

The Flickering of a Dying Flame

INGOLF LAMPRECHT & BERND SCHAARSCHMIDT

Zentralinstitut für Biochemie und Biophysik der Freien Universität Berlin

This article could well have been the basis of Michael Faraday's seventh lecture on the chemical history of a candle.

AROUND the Christmas of 1860 Michael Faraday gave his famous six lectures on the chemical history of a candle. These might well be looked on as the archetypes of the method of "teaching by examples", and for more than a hundred years the little book in which they were published¹ has kept his place in the scientific literature of many languages.

As Faraday had no experiences of Christmas trees he may never have observed the dying moments of a light on the tree and the flickering which accompanies its extinction. This rhythmical flaring, so very well known to us now, often has quite a loud noise associated with it. (Similar oscillations occur in many other contexts, in systems involving enzymatic regulation as well as in mechanical devices.)

We therefore thought it would be interesting and amusing to do some experiments on the regulation of the burning of a taper flame—the seventh lecture, so to speak. For this purpose we constructed a small triangular arrangement with a hole for the wick at the top and a pan beneath. And as a candle is not always made of wax and as Faraday himself used benzene in some of his demonstrations, we selected a few liquids that burn well, some of which are in fact used in lamps today. Our list comprises hexane (boiling point 68.8° C), ethanol (78.3° C), octane (105° C), xylol (140° C), aniline (184° C), butandiol (207° C), benzene (~120° C) and petroleum (~200° C). To follow the rather rapid flickering of flames we combined a photoelement with a recorder having a high chart speed of 60 cm min⁻¹. With this simple experimental arrangement we measured the frequency, the duration, and the number and form of the oscillations as functions of the length of the wick.

We first measured the length of the wick as accurately as possible, then immersed the whole of it in one of the liquids for a short time, poked it through the hole in the top of the triangular arrangement and lit it. The yellow colour of the flame soon changed to blue and then back to yellow again—it then began to flicker. Vertical oscillations started at the bottom of the flame whereas the top remained almost motionless all the time—at most it may just wander around the upper surface of the wick. As time passed the average brightness of the flame decreased more and more with perhaps occasional spectacular increases just before it disappeared.

There was often not only one continuous period of flickering but a group of several, divided by times of quiet burning. The highest number of groups we observed was eleven, for xylol.

As Faraday pointed out to his audience, the heat of the flame cannot vaporize all the liquid fuel on the surface of a wick. The bigger the flame, however, the quicker the fuel is used up. If the quantity of liquid transported to the top by capillary forces is smaller than the amount consumed the flame will diminish, generating less heat and therefore evaporating less fuel. In this way oscillations can arise as a direct consequence of the difference between the rates of transport and consumption of the fuel.

The first astonishing result of our experiments is that there

was only a very small range of wick lengths for which flickering occurred. With a wick 2.5 mm in diameter, as normally used for lighters, we saw no oscillations for wick lengths less than 1.5 mm and greater than 5.0 mm. Above this range the flame disappeared with one final increase in brightness and size; it died out slowly at wick diameters less than 1.5 mm. But within this range flickering always occurred for a considerable time, the length of which depended on the substance used. There seemed to be a specific threshold of brightness below which the flickering started.

The frequency of flaring did not stay constant during the whole flickering period but decreased or increased monotonically. Often a second slow oscillation of about 1 cycle a second was superimposed on the faster one.

The highest frequency observed with the substances listed previously was 23 cycles a second, the lowest about 7 cycles a second. The largest values were always found with the shortest wick, the frequency decreasing with increasing length. This result is demonstrated (together with the more classic experimental conditions) in Fig. 1; the frequency F is a linear function of the wick length W for benzene, whereas the period of oscillation T shows a pronounced maximum at about 2.5 mm. These graphs are similar for all the substances examined but differ in the slope of the frequency curve, the maximal oscillation time and the wick length at which the maximum occurs.

The oscillatory phenomena are due to capillary forces, which can be compared for the different liquids in terms of



Fig. 1 The authors² observing the frequency F (s⁻¹) of oscillations and the time of flaring T (s) for various wick lengths W (mm) for benzene.

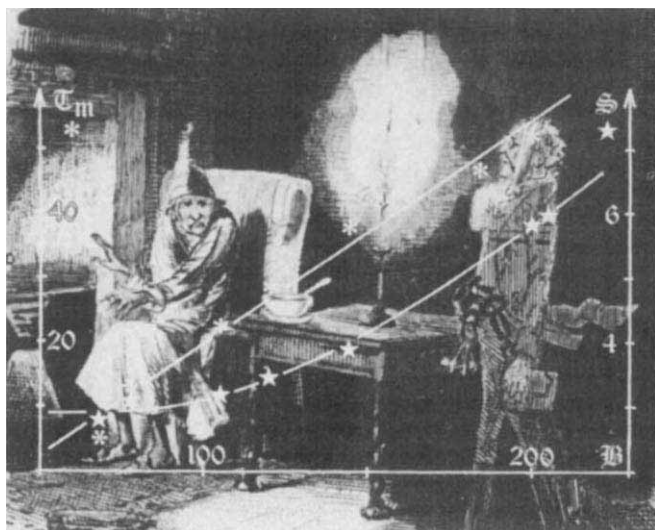


Fig. 2 The authors² discussing the behaviour of the maximal time of flaring T_m (s) and the slope S (s^{-1}/mm) of the curve of frequency against wick length with the boiling point B .

their surface tensions. As these are hard to measure we examined how the flaring characteristics varied with the boiling points (which are directly related to the surface tension).

It was not the frequency itself, but the slope S of the curve of F against W and the maximal time of flickering T_m , that we found to grow larger with increasing boiling point B , as demonstrated in Fig. 2. The same dependence holds for that wick length at which T_m occurs.

We found that very viscous fluids such as 3-chlor-1,2-propandiol burn brightly and steadily, indicating that the relationships implied in Fig. 2 are not only functions of the surface tension and boiling point, but of other parameters too.

The highest frequencies are combined with the smallest amplitudes of flaring and *vice versa*. At the very end of the period of flickering we often saw some slow oscillations (about 7 a second) with very large fluctuations in brightness.

We shall purposely pass no comment on the details of the flickering of a candle. Perhaps readers will be inspired to have a scientific look at their Christmas trees in order to confirm or reject the statements we have made.

¹ Faraday, M., *A Course of Six Lectures on the Chemical History of a Candle* (Griffin, Bohn, London, 1861).

² Dickens, C., *Christmas Books* (The Nonesuch Press, Bloomsbury, 1937).

The Problem of Reduction in Biology

JUNE GOODFIELD

Tile Barn, Alfriston, Sussex

Dr Goodfield sums up a conference that was held at the Villa Serbelloni, Bellagio, Italy, between September 8 and 16.

CONFERENCES which bring together a wide variety of people with wide ranging interests are traditionally characterized by mutual cross-purposes and general irritability, especially when these people are philosophers and scientists. But at the beginning of September, Professors T. Dobzhansky and F. Ayala (University of California at Davis) cheerfully ignored earlier trends and, aided by the Rockefeller and Alfred Sloane Foundations, convened such a gathering with a historian of biology and a psychologist thrown in for good measure. This international group was invited to examine an old problem in biology and to do so across the entire biological spectrum, from immunology through evolution to the realms of brain and consciousness. One feared a curate's egg. The fact that for most of the time the conference was not only good but, in parts, very good indeed, has a great deal to do with the calibre of the participants, the ambience of Serbelloni, and the fact that the discussions went on for six days. In the end there were neither conclusions nor solutions, nor were there Cassandra-like utterances about the state of the world combined with pious exhortations for the future. A group of enormous versatility, with many interests and preconceptions, hammered away with humour and respect. The result was, as Professor Sir Peter Medawar (Clinical Research Centre, Harrow) phrased it, "Operation Re-think".

From the very beginning there was a wide divergence of opinion about the very notion of reduction itself. Three historically oriented papers, by Professor G. Montalenti (Citta Universitaria, Rome), Professor E. Boesiger (Laboratoire de Genetique, Montpellier), Dr. J. Goodfield (Michigan

State University and University of Sussex), followed by two technical ones by Professors Medawar and Edelman (Rockefeller University), highlighted the deviations which were immediately aired in discussion. Is reductionism essentially a problem of differing world views (Sir Karl Popper); a research stratagem (Professor Medawar); one of justification rather than discovery (Professor Edelman); an attitude which served only to focus on certain problems as being genuinely biological (Dr Goodfield); a problem of all human existence (Professor H. Skolimowski, Ann Arbor); or only how the true propositions of one science can be logically deduced from another (Professor D. Shapere and most philosophers)? All these aspects emerged from the wide ranging paper presented by Professor W. H. Thorpe (University of Cambridge).

The actual methods, problems and procedures of the "man in the lab" were competently presented by Professors Medawar and Edelman, but if the problem only amounts to one of research stratagems—a question of the value of a methodological procedure—then the issue is a straw one. Under the conference's concentrated gaze, the problem of reduction faded away like the Cheshire Cat until nothing remained but the smile—the smile of people with confidence in their methodology and optimistic of success. But as Professor Edelman was at great pains to point out, this external attitude of confidence could be entirely misleading. While pressing the distinction between praxis and understanding, between skill and comprehension, he also urged the conference to distinguish between the public manners of certain reductionists and their private procedures when confronting difficult biological problems. Humility in the face of a staggering complexity is, he argued, part of the stance—believe it or not. Both Professors Edelman and Medawar consistently emphasized that no one worth his scientific salt is tempted into over-simplistic approaches or solutions. Professor J. Monod (Institut Pasteur) later expressed the point most felicitously: a great scientist combines a genuine sense of humility with a capacity to make

audacious forays into theory from time to time.

The scientists were pressed hard on one point, which repeatedly comes up whenever the word reduction is mentioned. This is usually expressed by the aphorism that "the whole is more than the sum of its parts". Professor Medawar tackled this one while at the same time trying to remove the sense of mystery from the notion of emergence. The phenomena of biology appear only because of the progressively restrictive conditions under which they can exist, and these restrictions are the price paid for conceptual enrichment, which both limits and defines the subject matter. The biologist alternates between the strictly analytical view and wider considerations, moving up and down between the molecule, the cell, the organism, as both the situation and theory demand. Professor Edelman, indeed, told a cautionary tale at this point. The instructive theory of antibody formation turned out to be incorrect, and its failure, he argued, rested not in its reductionist methods, but because the investigators approached the problem only at one level, namely that of the molecule rather than the cell. Biologists chip away at complex problems how and when they can, using every tool to hand, technical and theoretical. But the fact that a problem is complex and has not yet been solved is, by itself, no evidence that it cannot be done. Here the question of reduction remains an open one; there can be no *a priori* axiom of impotence.

It was inevitable, too, that the problem of "different" and "unique" forms of biological concept should be raised. But the absence of similar concepts in physics and chemistry could be for several reasons. The problems that these concepts were designed to tackle might be neither relevant nor interesting to a physicist. Moreover, if only a limited class of possible interactions constitute the subject matter for one discipline—for example, economics—then the notion of, say, a foreign trade deficit (Professor Medawar's favourite example) will have no place. But when physics and chemistry do encounter similar kinds of problems to biology, then similar forms of concept emerge and the subject expands like a concertina to embrace them. The examples were revealing. Polymer chemistry now deals with time-reversal phenomena. The subject matter of chemistry has not until quite recently required chemists to link thermodynamics to a principle of selection; physics is now facing problems of hierarchies of organization long familiar to biologists; physicists are also interested in such phenomena as the changes in the qualities of the varnish on a Stradivarius violin, these being relaxation phenomena demonstrating both temporal and irreversible properties. Yet, nevertheless, as Professor Monod later reminded his colleagues at the conference, when the group of physicists, of whom Delbrück is perhaps the most well known, moved over to phage phenomena, they had to become biologists in a very real sense. The physics then existing was no longer their subject matter. One sensed an air of relaxed agreement at this point. We were all methodological reductionists at heart, and confident ones at that.

Into this quiet pool of satisfaction Professor Skolimowski proceeded to drop a stone; it would be unfair to call it a brick. In his paper on rationality and biology, he proceeded to call in question the very methodology on which everyone had just agreed and to do so not only within biology but within the whole of science. He invited us to discard what he termed "science's ideological strait-jacket", and his tones were most reminiscent of the radical student left. The faces of the practising scientists when told to "break free from the chains of conceptual tyranny that had enslaved them for years" were a study. Professor Skolimowski's point—one among many, many others—seemed to be this: the tradition of scientific enquiry has gone on for so long that no one is prepared to admit, even as a possibility, that there might be other methods—other means of acquiring knowledge—equally valid and equally useful. A recognition of this might assist an understanding not only of areas at present

outside science but also of some problems currently within science. When the conference had drawn its collective breath, he was given a particularly thorny problem in immunology and invited to tell us how to proceed from there on. For it was not, the scientists insisted, as if they were too proud to accept help. Their problems were so complex that, short of a crystal ball, they would accept solutions from whatever source. But Professor Skolimowski was not, one felt, convinced of the sincerity of their affirmations, nor were the scientists of his capacity to help. This paper was charmingly provocative, presented with passion and conviction, and was argued with high old humour by all—good, clean fun.

Not all the philosophical papers were so subversive, though the one by Professor B. Rensch (Westfälischen Wilhelms Universität) took the conference into the dark speculative waters of panpsychic phenomena. As Professor Medawar had tried to take the mystery out of the notion of emergence, so too did Professor M. Beckner (Pomona College, California) for the notion of hierarchies, and he emphasized once again that the limiting factors on conceptual extension throughout the sciences might simply be those of relevance and interest. Professor Shapere, who consistently pulled the conference back to the chief problem whenever it seemed to wander, forced again the distinction between methodological reductionism and the more formal definitions given by philosophers of science. He examined the "problems of problems", asking how it is that certain facts come to be associated together and so require explanation, and why some lines of research appear more promising than others from the very outset. The study of such questions increased our understanding of scientific method and Professor Beckner went on to argue that this was all that a study of reduction could do; namely, give an insight into the conditions and strategies of the application of one science to another. As for the consequences, or relevance, of such studies on the procedures of scientists, they would probably have no effect in practice—and why should they? In the context of the laboratory there were few discernible differences between Loeb, Edelman, Medawar, Monod and Crick on the one hand, and Bernard, Weiss and Lettvin on the other. And although Dr Goodfield, concentrating only on such practising scientists, attempted to demonstrate a genuine difference between reductionists and anti-reductionists, the substantive point remained elusive.

When the conference moved over to the area of evolution, methodological issues became subdued and more conceptual ones predominated. The contingent from Davis, California (Professors Dobzhansky, Ayala and Stebbins), presented three papers, beautifully constructed and articulated, in which the notions of chance, progress, creativity and novelty in evolution were carefully examined; they stimulated much discussion. Even Professor Monod permitted Dobzhansky to use the analogy of "engineer" and "artist" with regard to natural selection, as in some real sense a "putting-of-things-together", although he uttered a note of caution of the dangers as he did so. Professor Monod gave as an example present work in molecular genetics: the scientists reason out the properties of a possible mutant, then create an environment which encourages its appearance. "Engineer" and "creator" certainly are the operative words in this case. It seemed, however, that there was still a marginal difference between them; for Professor Monod information is acquired during evolution by a random process of erasing everything unusable, but one sensed that for Professor Dobzhansky it implied a more positive creation of order from disorder. Perhaps the distinction is really slim, although Stebbins wondered whether many difficulties might be avoided if instead of the words "natural selection" we consistently substituted the phrase "population-environment reaction".

The term natural selection was to raise the problem of reduction again. Is Dobzhansky's definition—the differential reproduction of living systems—a truly non-reducible concept? Edelman and Shapere regarded this question almost

as a personal challenge, and set out to produce examples of selective processes which could show that biological selection was just a special case of a more wide-ranging principle. A spectrum of selective processes had in any case been examined earlier in Professor Edelman's paper, which gave a neat technical account of a well-analysed approach to the problem within the immune system. But then Professors Edelman and Shaper proceeded to produce four delimiting criteria based on earlier definitions by Professor Stebbins. The first two served as a general notion of interactions, while the third distinguished "selection" as those interactions in which the environment played a role. Self-replication with mutation was their fourth criterion and this specified "natural selection". They argued that within contemporary astrophysics, the theory of star production from mass condensations of interstellar compounds, under the influence of environmental conditions, provided a genuine example of selection in a strict sense. There are certainly some selective systems that might prove difficult to classify; those of the immune system, where selection occurs among cells rather than organisms, and those examples provided by Spiegelman's Q-beta phage experiments. But nevertheless they questioned whether even the specified features of natural selection, as embodied in their fourth criterion, implied the impossibility of explanation in physico-chemical terms. For however improbable the event, the origin of life required the existence of molecules which could reproduce with mutation and which had a differential reproduction due to environmental interaction.

Professor D. Campbell (Northwestern University) was to extend the notion of variation and selection in another direction. Evolutionary types of explanation have been gaining ground over the past few years, providing models of scientific growth and theories of knowledge and also being used more generally within the history of ideas and sociology. Professor Campbell has for some time been one of the most effective proselytizers. In a long and exhaustive paper he attempted to apply the model both to creative thought and to scientific processes—in fact to all processes that might be termed "adaptive". His paper and presentation were stimulating and provocative, this time in the very strict sense of thought-provoking. Some people were doubtful about the possibility of applying the idea of natural selection to the world of ideas in any strict manner at all. In nature every possibility can come up; all can be tried. But in the cultural world, many ideas are simply not "idiotic" enough, as one scientist put it. For in the experimental situation there is already a preselection of intellectual variants which is determined by the sociological niche of the discipline, and only moderately sane ideas ever emerge. So the intellectual matrix in which we find ourselves not only determines but even shapes our ideas. This was reasonable enough, but, when Campbell went on to suggest that worship of the ecological niche had somehow supplanted worship of God, his argument became somewhat tortuous.

The problem of brain and consciousness provided the most forthright discussion and serious disagreements. It also provided the most unlikely alliances, with scientists and philosophers ranged against scientists and philosophers. The problem was first exposed by Professor C. Birch (University of Sydney), who said that, as a practising scientist, he pressed reductionist methods as far as they would take him but, at the point of feelings, understanding and consciousness—the most real things he knows—both the method and the explanations fail. Sir John Eccles (State University of New York, Buffalo) began from a similar point. Consciousness is the primary reality and, though as a neurophysiologist his methods are reductionist, yet reduction did fail, and would continue to fail, over the mind-body problem. His argument rested basically on three foundations: first, the sheer complexity of patterned operations within space and time, in the brain, and of the neuronal hook-up—all of which might defy analysis; second, on Sperry's split-brain experiments; and, third, on

the interaction of the world of the conscious self (World 2), with the world of the products of the human mind (World 3 of Popper).

Some of the criticism was stringent and came from both scientists and philosophers. Although the facts of the split-brain experiments are indubitable, is an interpretation that locates consciousness in one hemisphere really justified by the data? Is Eccles right to be so pessimistic about the potentialities of our existing methodology to tackle what Professor Monod terms the "second frontier"?—a pessimism shared neither by Professors Medawar nor Edelman. Even being called a thorough Cartesian failed to shake Sir John Eccles from his firmly held and forcefully argued beliefs. This label merely stimulated him to say stoutly: "I'll be reduced to physics and chemistry only when I'm dead!"

He had firm but gentle support from Sir Karl Popper, the philosopher with whom most scientists have a real empathy. Sir Karl argued with cogency that the problem of man's emerging consciousness is likely to be insoluble by biological and functional methods, and, drawing on the history of science for his examples, he tried to demonstrate that there has never in fact been a successful total reduction.

As a result of these discussions one thing became clear. It is trite but true. Whatever the respective tools that scientists, psychologists and philosophers bring to the study of brain and consciousness, an enormous amount of basic analytical work remains to be done. We will be chipping away at this problem, from all sides, for a long time yet, and it will be many years before we are able to build up a picture of any reasonable overall size. This also means, however, that this problem will continue to provide the basis for mutual cross-purposes, not only between scientists and philosophers but even among scientists themselves.

In the last session the conference extended its brief once again to the problems of value and knowledge in an objective world. There had been an air of "Waiting for Monod", and he arrived halfway through, neatly plugging the gap left by Professor Edelman's early departure. This means that the participants were never without the stringencies provided by at least two fully committed working reductionists, and if there was ever any temptation to float off into an intellectual empyrean these scientists pulled us firmly back. Everyone had read Professor Monod's book and many of the critiques too, so there was no need to question him about his views on reductionism. It was, however, a real deprivation that he had not been present for the discussions on consciousness. Critical pressures by Professor Skolimowski on Professor Monod's notions of objectivity, by Professor Thorpe on his notions of value, served, amongst others, to show that this whole area deserves a far more intensive discussion than the time allowed. The relation between a system of values and knowledge—relations which are either presupposed or dealt within most philosophies and religion—has never been given the fundamental shake-up it needs in the context of twentieth-century science. Professor Monod may have provoked a prejudiced hostility in some places, but for all the brevity of his thesis he may have been one of the first scientists to face squarely the implications of our present intellectual situation.

The discussions gave a real unity to the conference and they were, at one and the same time, amiable but tough. It is a great pity that they were not recorded and subsequently edited, even though one hesitates to put extra burdens on overworked organizers. The book that is to appear from the papers alone cannot do justice either to the quality of the argument, or to the open-mindedness of people's attitudes or to the degree of sophistication that finally developed around the notions of reduction. For too long people have assumed that there was only one meaning—and that was the one they were using. But in future anyone who allows the word to slide off his tongue in a glib and loose fashion will simply not be up to date—and what could be more unfortunate?

A Possible Role for Histone in the Synthesis of DNA

HAROLD WEINTRAUB*

Department of Anatomy, University of Pennsylvania, Philadelphia, Pennsylvania 19104

When protein synthesis is inhibited, DNA synthesis continues normally but with a slower rate of chain elongation. Dr H. Weintraub speculates that the histones are the DNA chain elongation proteins.

WHEN protein synthesis is inhibited in primitive chick erythroblasts, linear DNA synthesis continues for about 45 min, but at half the control rate¹. The depression in DNA synthesis occurs within 25 s of the addition of cycloheximide, is readily reversible, and is manifested as a decrease by half in the gross rate of DNA chain elongation. The labelled DNA made under these conditions is covalently bound to previously synthesized DNA and associated with single stranded segments well over 10⁷ molecular weight. The effects of cycloheximide on DNA synthesis are due neither to a direct action on the DNA replicase, nor to an effect on cell permeability or nucleotide metabolism mediated by decreased protein synthesis. It is also unlikely that this behaviour is a consequence of the accumulation of an inhibitor in the absence of protein synthesis¹. All our data indicate that when the synthesis of protein is inhibited, the synthesis of DNA proceeds normally except that the rate of chain elongation is slower. This applies to each replicon in each S phase cell. Here I present evidence that the proteins, termed chain elongation proteins (CEP), involved in this fine control of DNA synthesis are histones.

Cycloheximide Resistance

It was previously shown¹ that low concentrations of cycloheximide or puromycin, although inhibiting bulk protein synthesis by as much as 30%, resulted in no inhibition of DNA synthesis. This could indicate that the synthesis of the CEP was relatively resistant to low concentrations of inhibitor, or that a pool of this protein existed within the cell. The latter possibility was unlikely since higher concentrations of inhibitor lead to a maximal effect on DNA synthesis within 25 s. The dose response curves to cycloheximide for DNA synthesis and the synthesis of various protein fractions are shown in Fig. 1. DNA synthesis was linear for at least 30 min. The curve for DNA synthesis normalized to the maximum amount of DNA inhibition is also given. Data show that histone synthesis is relatively resistant to cycloheximide^{3,4} and that the dose response curve of histones follows the normalized dose response curves for DNA synthesis. SDS-acrylamide gel analysis of the cytoplasmic fraction and the acidic nuclear protein fraction at 1, 5, and 20 μ M cycloheximide detected no proteins resistant to cycloheximide using a double-label analysis. Similar experiments showed all histones to be equally affected at each cycloheximide concentration.

* Present address: MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England.

The Effects of Amino-acid Analogues

Compared to other proteins, histones are unusual in that they contain no tryptophan, little tyrosine, but much arginine. It is shown in Table 1 that DNA synthesis is (a) resistant to high concentrations of the tryptophan analogues, methyl-tryptophan and beta indole acrylic acid, (b) only slightly sensitive to high concentrations of the tyrosine analogues, methyl-tyrosine and iodo-tyrosine, and, (c) extremely sensitive to low doses of the arginine analogue, canavanine. Also that the tryptophan and tyrosine analogues can interfere with the metabolism of their corresponding amino-acids as equimolar concentrations can markedly depress uptake of labelled amino-acid into TCA precipitable material. Addition of cycloheximide with canavanine results in only a slight potentiation. This indicates that both drugs are probably affecting the same step in DNA synthesis, made more likely by the observation (not shown) that the kinetics of inhibition by canavanine mimic those of cycloheximide. In canavanine, ³H-leucine incorporation is decreased by 10%. (Fig. 1 shows that a comparable amount of inhibition by cycloheximide has no effect on DNA synthesis.) This might indicate that canavanine affects DNA synthesis by inhibiting the synthesis of a specific class of

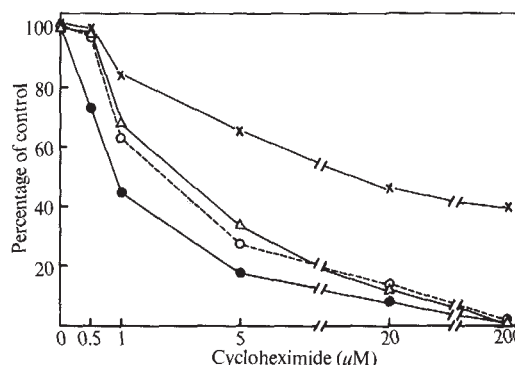


Fig. 1 Resistance of histone synthesis to cycloheximide. Four day old erythroblasts were incubated *in vitro*¹ in L-leucine-4, 5-³H (20 μ Ci ml⁻¹; 35 Ci mM⁻¹) or ³H-methyl-TdR (20 μ Ci ml⁻¹; 15 Ci mM⁻¹) for 30 min in the presence and absence of the stated concentrations of cycloheximide. The various protein fractions were then isolated² from cells labelled with leucine. Briefly, nuclei were obtained from washed cells using 0.5% 'Nonidet' in RSB (0.01 M NaCl; 0.01 M Tris-HCl, pH 7.4; 0.003 M MgCl₂). Histone (○---○) was extracted twice from washed nuclei using 200 volumes of 0.25 M H₂SO₄. The residual proteins were termed the acidic nuclear proteins. DNA (x—x), added to the cytoplasmic fraction, sedimented and extracted with acid (●—●), failed to show any bound histone-like protein. The incorporation is expressed as a percentage of that incorporated by control cells either by an internally controlled double-label analysis² or by direct measurements. Incorporation of ³H-TdR into TCA precipitable material was measured directly¹. "Normalized DNA" (△—△) represents the amount of inhibition by cycloheximide as a percentage of the maximum amount achieved at 200 μ M (68% inhibition). All isotopes were obtained from New England Nuclear Corporation; all analogues and inhibitors, from Sigma Corporation. Radioactive incorporation was measured using a 'Beckman LS-200 Liquid Scintillation Counter'.

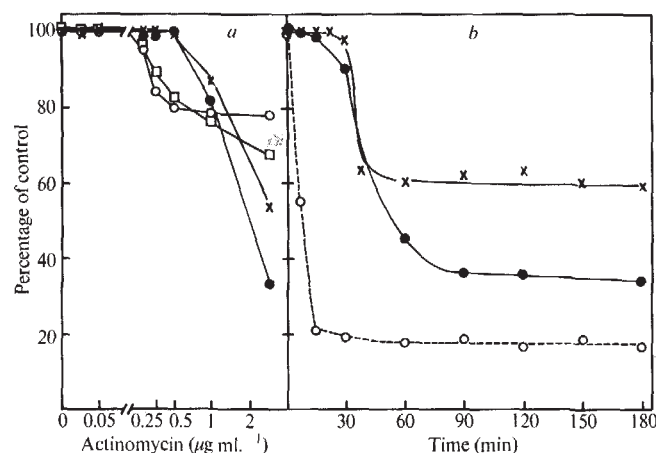


Fig. 2 *a*, Resistance of histone synthesis to low concentrations of actinomycin D. After 90 min incubation in actinomycin D at the stated concentrations, the cells were labelled with ^3H -leucine ($20 \mu\text{Ci ml}^{-1}$) for 30 min; the various protein fractions isolated; and the incorporation compared to control cells, either directly, or using double-label methods. $\bullet$ — $\bullet$, Histone; $\times$ — $\times$, DNA; $\square$ — $\square$, nuclear acidic proteins; $\circ$ — $\circ$, haemoglobin. *b*, Kinetics of incorporation after $2.5 \mu\text{g ml}^{-1}$ actinomycin D. Incorporation of ^3H -TdR ($10 \mu\text{Ci ml}^{-1}$), ^3H -5-uridine ($10 \mu\text{Ci ml}^{-1}$ 20 Ci mM^{-1}), and ^3H -leucine ($20 \mu\text{Ci ml}^{-1}$) into histone was measured as a rate percentage of controls after addition of actinomycin D ($2.5 \mu\text{g ml}^{-1}$). $\times$ — $\times$, ^3H -TdR; $\bullet$ — $\bullet$, ^3H -leucine in histone; $\circ$ — $\circ$, ^3H -U.

proteins, rather than producing altered proteins of a specific class.

Actinomycin Resistance

Fig. 2*a* shows the dose response for DNA synthesis and the synthesis of the various protein fractions after a 90 min pre-incubation with actinomycin D⁵⁻⁷. Both histone and DNA synthesis are again comparatively resistant to low concentrations of inhibitor, both follow curves compatible with those obtained using cycloheximide. Fig. 2*b* gives the time course for the inhibition of histone, DNA, and RNA

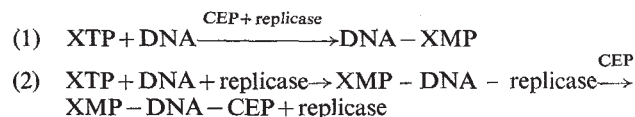
synthesis. After a lag of about 30 min, both histone and DNA synthesis decrease to a constant rate. Aside from this lag period the kinetics for both DNA and histone synthesis are similar to those obtained from cultures inhibited with sub-maximal doses of cycloheximide ($5 \mu\text{M}$). In actinomycin at $2.5 \mu\text{g ml}^{-1}$, RNA synthesis is about 18% of controls and constant by 5 min. If actinomycin had been affecting DNA synthesis directly, presumably by binding to the DNA, it might have been expected that DNA and RNA inhibition follow the same time course. This is clearly not the case. Additional experiments have shown that pre-incubation with actinomycin ($2.5 \mu\text{g ml}^{-1}$) fails to add to the inhibition of DNA synthesis caused by cycloheximide. This supports the idea that both actinomycin and cycloheximide are affecting the same protein species. Although it is possible that both drugs are inhibiting the synthesis of an RNA species necessary for chain elongation, it becomes somewhat less likely since in both cases bulk RNA synthesis follows a different time and dose dependence than DNA synthesis.

Relation to DNA Synthesis

It was previously shown¹ that after release of FUDR blockage of DNA synthesis, there is a lag in the onset of inhibition of DNA synthesis by cycloheximide. The lag period increased with increasing exposure to FUDR, but the length of the lag was extremely short compared to the length of exposure, for example, 10 h in FUDR resulted in a lag time of about 20 min. The lag period was interpreted as indicating the accumulation of a pool of chain elongation protein. The disparity between the exposure to FUDR and the length of the lag could indicate either of two processes; (a) chain elongation protein is rapidly synthesized and partially degraded, or (b) the synthesis of chain elongation protein is coupled to the synthesis of DNA, but the coupling is leaky.

Synthesis of nuclear histone is sensitive to inhibition by cytosine arabinoside^{6,7} as shown in Fig. 3. A similar curve is obtained for FUDR. At high levels of DNA inhibition, histone synthesis is not completely inhibited; thus histone synthesis is coupled to DNA synthesis in these cells in a leaky fashion. Acrylamide gel analysis has demonstrated that although the synthesis of all histone species is inhibited, the slightly lysine-rich histones are somewhat more sensitive⁸. Attempts to detect other proteins, cytoplasmic and nuclear acidic, which follow either of the two criteria listed above have not yet been successful.

Given these results, it is possible to ask if CEP participates in the same step of chain elongation as deoxynucleotide addition. Two possibilities can be schematized as follows:



If TTP is made rate-limiting with FUDR such that the overall rate of precursor incorporation is some 10–30% of controls, then, given the results described in the previous paragraph, the synthesis of the CEP would also be inhibited. If deoxynucleotide addition and the CEP share the same step (1), then an initial inhibition of DNA synthesis should eventually lead to a subsequent inhibition of DNA synthesis because the concentration of two participants (TTP and CEP) of a multi-molecular reaction would then be decreased. Moreover, this process is auto-catalytic and the rate of DNA synthesis should gradually drift toward 50% of the control rate. This is the amount of DNA synthesis not dependent on concurrent protein synthesis. On the other hand, if deoxynucleotide addition and CEP participate in two separate reactions (2), the initial amount of inhibition should be stable, and a drift toward 50% inhibition should not be

Table 1 Effect of Amino-acid Analogues on the Synthesis of DNA

Treatment	Amino-acid ratio ⁴ (histone to nuclear acidic)*	Ratio of analogue to amino-acid†	% Inhibition of ^3H -TdR incorporation	Ratio of analogue to amino-acid†	% Inhibition of labelled amino-acid uptake‡
C- ^3H -tryptophan	0	100 : 1	0	1 : 1	22%
3β -Indole acrylic acid	0	100 : 1	0	1 : 1	24%
3-Iodo-L-tyrosine	0.09	100 : 1	22%	1 : 1	25%
O-C- ^3H -L-tyrosine plus cyclo.	0.09	100 : 1	24%	1 : 1	26%
Cyclo. alone			56%		
L-Canavanine	1.83	1 : 1	53%		
plus cyclo.			62%		
Cyclo. alone			68%		
			58%		

Cells were treated with the analogues and labelled concurrently with ^3H -TdR for 30 min.

* Ratio of amino-acid in histone to that in nuclear acidic protein for the amino-acid corresponding to the given analogue. Incorporation of ^3H -TdR was measured as a percentage of an untreated control culture.

† Ratio of analogue to amino-acid in the medium.

‡ A 1 : 1 ratio of analogue to amino-acid was tested for its effect on the incorporation of labelled amino-acid for 30 min into TCA precipitable material. L-tryptophan- ^3H (G) (3 Ci mM^{-1}) was at $5 \mu\text{Ci ml}^{-1}$ and L-Tyrosine-3,5- ^3H ($25 \mu\text{Ci mM}^{-1}$) was at $10 \mu\text{Ci ml}^{-1}$. In all cases background was obtained from a sample taken at time zero. To test for effects of the analogues at levels other than protein synthesis, cycloheximide ($20 \mu\text{M}$) was added alone and together with the analogue and the degree of inhibition monitored.

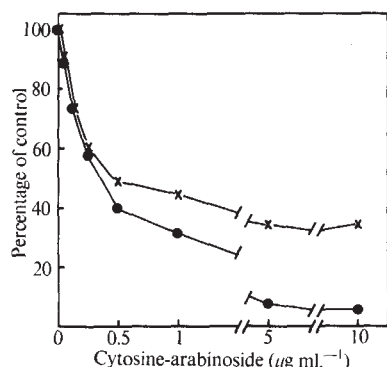


Fig. 3 Leaky coupling of histone synthesis to DNA synthesis. After 90 min in the stated concentrations of cytosine arabinoside, control and treated cells were labelled with ^3H -TdR ($20 \mu\text{Ci ml}^{-1}$) and ^3H -leucine ($25 \mu\text{Ci ml}^{-1}$) for 30 min. The inhibition of DNA (●—●) and histone (×—×) synthesis as a function of dose was measured as described in Fig. 2.

observed. If CEP is histone, Fig. 3 shows that both steps of the overall reaction depicted in (2) are inhibited to about the same extent. Either one becomes rate limiting and no secondary decreases in the overall reaction rate will occur. Experiments designed to distinguish between these two possibilities favour the second mechanism. With either FUDR or cytosine arabinoside it was found that various low levels of inhibition were stable over at least 3–4 h. These experiments measured the incorporation of ^3H -deoxyadenosine into alkali stable, TCA precipitable material a high concentration ($10 \mu\text{g ml}^{-1}$) of deoxyadenosine in the medium was used to diminish effects from small fluctuations in the internal pool size. The acid soluble pool was monitored for all time points, and the precipitable incorporation adjusted accordingly. Similar conclusions could be derived from analogous experiments using labelled thymidine and deoxycytidine. I conclude that the process of DNA chain elongation *in vivo* is separable into at least two steps and that the step sensitive to decreased levels of CEP is different from that responsible for nucleotide addition.

A Presumptive Replication Complex

Pulse labelled DNA in erythroblasts displays characteristics which are similar to those described in HeLa cells^{9,10}. Nascent DNA is preferentially extracted in either phenol or chloroform-isoamyl alcohol and also sediments to the top of a CsCl gradient. This behaviour, which probably indicates the association of nascent DNA with a low density, hydrophobic material, is, for erythroblasts, resistant to former treatment with pronase SPS, high salt, RNAase, or periodate, but sensitive to shear, sonication, alpha-amylase and phospholipase C (Sigma). "STS" gels have demonstrated contaminants in both of these last two enzyme preparations. It would be premature, therefore, to define the complexing agent in terms of either phospholipid or polysaccharide on the basis of these enzyme sensitivities. When pure DNA is incubated with either of these enzymes neither a loss in TCA precipitable counts per min nor a decrease in molecular weight in alkaline sucrose gradients could be detected. Because denatured DNA is preferentially extracted with chloroform¹¹, and as pulse-labelled DNA might be partially denatured, reconstruction experiments were done in which labelled, denatured DNA was added to nuclei before treatment. These experiments showed that although the labelled, denatured DNA was preferentially extracted with chloroform, it sedimented to a higher density than native DNA on CsCl and that both of these characteristics were unaffected by shear, alpha-amylase, phospholipase C, or sonication and denaturation. It is therefore unlikely that the behaviour of pulse-labelled DNA in erythroblasts results only from some single-stranded property. Most evidence indicates that pulse-

labelled DNA is associated with some nuclear structure which confers certain physical properties on it.

Fig. 4a shows the percentage of water soluble counts (counts not extracted with phenol) after a pulse of ^3H -TdR (5 min) plotted as a function of the chase time in cold medium. Graphs are given for chases in the presence and absence of cycloheximide. Addition of the drug during the pulse period makes little difference. Care was taken to expose the DNA to the same amount of shear for each point. Better separation of the complex is obtained if, instead of extracting the DNA, it is sedimented to equilibrium on CsCl (4b). The kinetics displayed in Fig. 4a and b are consistent with the model proposed by Friedman and Mueller⁹ where the extractability of pulse-labelled DNA is a function of the distance of that DNA from an extractable replication complex. The differing behaviour of cycloheximide treated cells is then explained by a slower rate of DNA chain elongation during the chase period. Recent findings make it unlikely that this complex is associated with the nuclear membrane¹¹.

Histones Remove Nascent DNA from the Replication Complex

The effect of added whole calf thymus histone (Sigma) on the extractability of nascent DNA is shown in Fig. 5. Cells were pulsed for 10 min with ^3H -TdR and nuclei isolated in RSB these nuclei do not incorporate ^3H -TTP. The nuclei were then suspended in increasing concentrations of histone, albumin, or haemoglobin and incubated at 37°C for 30 min. SDS was then added to 0.1% and the DNA extracted with chloroform-isoamyl alcohol. Depending on

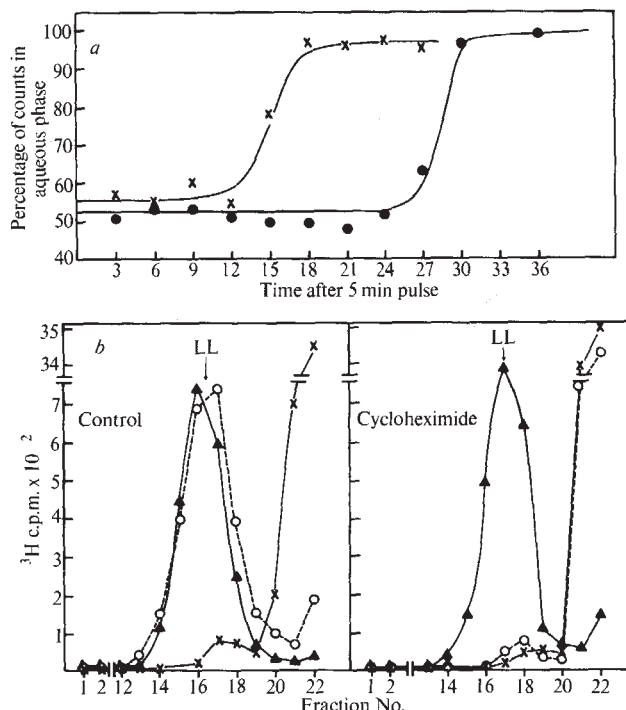


Fig. 4 Chasing of nascent DNA from a presumed replication complex in the presence and absence of cycloheximide ($50 \mu\text{M}$). Cells were pulsed for 5 min with ^3H -TdR ($50 \mu\text{Ci ml}^{-1}$) and chased for increasing periods of time in the presence or absence of cycloheximide. a, The isolated nuclei in RSB were treated with SDS to 0.1%. An equal volume of phenol was added and the suspension mixed on a 'Vortex' for 30 s. After centrifugation in a bench centrifuge, the water phase was removed and precipitated with TCA (10%) and carrier and then counted. ×—×, Control; ●—●, cycloheximide. b, The SDS treated nuclei were sedimented to equilibrium on CsCl¹ after a 30 s agitation on a 'Vortex' mixer. Elimination of SDS fails to affect either the extraction or the sedimentation results. LL, Native DNA. ×—×, 5' pulse; ○—○, 10' chase; ▲—▲, 40' chase.

the experiment, 5 to 50 $\mu\text{g ml}^{-1}$ of histone could protect over 90% of the nascent DNA from extraction. This protection was inhibited by 2 M NaCl before boiling the histone solution (5 min) and by exogenous DNA; haemoglobin and albumin were ineffective. Treatment of nuclei with histone before SDS solubilization also removes nascent DNA from the complex that sediments to the top of a CsCl gradient (Fig. 5, inset). High histone concentrations consistently inhibit the reaction with the replication complex. When used in high concentrations (protamine and cytochrome *c* have activity in this assay. Both are about 20% as effective as either whole histone or the lysine-rich histones when used at low molecular concentrations. Spermine, spermidine, and putrescine at concentrations up to 10^{-3} M fail to dissociate the complex and also fail to relieve the cycloheximide inhibition of DNA synthesis in the intact cell. The effective concentration of histone in these experiments is about the same as that in the cell, although this is a difficult comparison since most cellular histone is bound to DNA. Preliminary results indicate that over 50% of the exogenous histone becomes sequestered in nuclei during the course of experiments similar to those in Fig. 5, and that this histone can protect the nascent DNA from degradation by pancreatic DNAase. Similar experiments to be presented in more detail at a later date have shown that compared to chase DNA, pulse DNA is relatively resistant to DNAase and that this resistance is destroyed by an α amylase preparation that has no effect on the digestion of chase DNA by DNAase.

I conclude that *in vitro* the proper concentration of whole calf thymus histone can remove nascent DNA from a replication complex. How this activity might relate to DNA synthesis in the intact cell remains to be seen. Other basic proteins have activity in this assay but the histones are much more active at lower molar concentrations. Results with the replication complex are consistent with a previous conclusion that the CEP acts at a step other than nucleotide addition. They are also related to recent data showing that (1) histone stimulates Q β replicase *in vitro*; (2) bacteria grown in the presence of canavanine accumulate or fail to remove their DNA from complex¹³; and (3) ϕ X capsid proteins are required for ϕ X DNA synthesis¹³.

Probable Role of Histones

None of the experiments that I have presented proves that the histones are the DNA chain elongation proteins, although five independent experiments *in vivo* and *in vitro* make this notion likely. Final proof would be the demonstration that the proper concentration histone increases the rate of chain elongation in a cell-free system to that observed *in vivo* and that newly-made histone is located at the growing point of replication in intact cells.

There are several other reasons why the histones are a reasonable candidate for the chain elongation protein. First, they are found in the nucleus bound to the DNA. Second, consistent with the rapidity of inhibition of DNA synthesis by cycloheximide, they are usually not found in any detectable free pool. Third, given the preceding observations it follows that the chain elongation protein must move rapidly from its presumed site of synthesis on cytoplasmic ribosomes to the growing point. It is at least questionable whether diffusion can account for the rapidity of this interaction which must involve recognition as well as transport. The electrostatic forces between the newly polymerized DNA phosphates and the basic histones might facilitate this process. Fourth, there are some 3,000 growing points in the average S phase cell¹. Inhibition of DNA synthesis by cycloheximide probably occurs within 10 s. The rapidity of inhibition, the linear kinetics after inhibition, and the fact that low levels of cycloheximide give low levels of DNA inhibition indicate that the CEP is probably used stoichiometrically. As our previous experiments showed

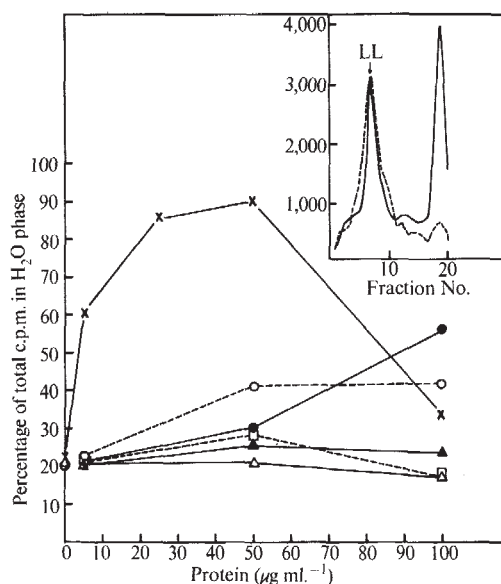


Fig. 5 Removal of nascent DNA from a replication complex by histone. After a 10 min $^3\text{H-TdR}$ ($25 \mu\text{Ci ml}^{-1}$) treatment, nuclei were isolated from cells and incubated in RSB at 37°C for 30 min in the stated concentrations of protein under the indicated conditions. Nuclei were then treated with SDS and extracted with chloroform-isoamyl alcohol as described in Fig. 4a for phenol extraction. The percentage of the total incorporated counts associated with the water phase was then determined. In representative experiments, all of the extracted counts could be recovered in the non-aqueous phase. Control experiments showed that the extractability of nascent DNA from nuclei lysed in high salt was not dependent on the presence of SDS and that added single-stranded DNA remained at the interphase despite the prior addition of histone. $\times-\times$, Histone; $\circ---\circ$, heat denatured histone; $\bullet-\bullet$, histone and 25 μg DNA; $\blacktriangle-\blacktriangle$, histone and 2 M salt; $\triangle-\triangle$, haemoglobin; $\square---\square$, BSA. Inset shows the sedimentation behaviour on CsCl of nascent DNA derived from untreated nuclei and nuclei treated with 25 $\mu\text{g ml}^{-1}$ of histone. By lowering the CsCl concentration compared to that shown in Fig. 4b, the complex is seen to move into the gradient. In all experiments, nuclei were at a concentration of about 10^7 ml^{-1} . SDS gel electrophoresis showed that the histone preparation contained one minor and one major contaminant (%). The preparation also contained no exonuclease activity; endonuclease activity was not monitored. —, Control; ---, histone. LL, native DNA.

that all replicons were affected, it follows that at least 20,000 CEPs must be synthesized by the cell per minute in order to sustain the normal rate of replicon growth. These cells make about 40,000 histone molecules per minute²; no other protein in the cell, apart from haemoglobin, even approaches this synthetic rate.

The most intriguing feature of the inhibitory effects of cycloheximide on DNA synthesis is that about 50% of the DNA can be replicated. Persistence of this activity can be explained if histone from pre-replicative chromatin acts as a reservoir of histone at the growing point. In conjunction with newly synthesized histone, the reaction of this recycled histone with nascent DNA and the replication complex allows the growing fork to move ahead at the normal rate. In the absence of newly synthesized histone, the rate of chain elongation is slower, but not zero since recycled histone can still be used.

Because all known polymerases have the inherent property to elongate nascent chains, the proposed requirements for histones in this process most probably reflects some higher order mechanism by which a synthesis of new DNA is coupled to the generation of a very defined and inviolable chromosomal structure.

I thank H. Holtzer, J. Flaks, and A. Kozinski for their help and criticisms. This work was supported by grants from the National Institutes of Health, the United States Public Health Service, and the Helen Hay Whitney Foundation.

Received June 12; revised October 12, 1972.

- ¹ Weintraub, H., and Holtzer, H., *J. Mol. Biol.*, **65**, 13 (1972).
- ² Weintraub, H., Campbell, G., and Holtzer, H., *J. Mol. Biol.* (in the press).
- ³ Spalding, J., Kajiwar, K., and Mueller, G. C., *Proc. US Nat. Acad. Sci.*, **56**, 1535 (1966).
- ⁴ Malpoix, P., Zampetti, F., and Fievez, M., *Biochim. Biophys. Acta*, **182**, 214 (1969).
- ⁵ Freedman, M. L., Honig, G. R., and Rabinovitz, M., *Exp. Cell Res.*, **44**, 263 (1966).
- ⁶ Borun, T. W., Scharff, M. D., and Robbins, E., *Proc. US Nat. Acad. Sci.*, **58**, 1977 (1967).

- ⁷ Mueller, G. C., and Kajiwar, K., *Biochim. Biophys. Acta*, **119**, 557 (1966).
- ⁸ Sadgopal, S., and Bonner, J., *Biochim. Biophys. Acta*, **186**, 349 (1969).
- ⁹ Friedman, D. L., and Mueller, G. C., *Biochim. Biophys. Acta*, **174**, 253 (1969).
- ¹⁰ Pearson, G. D., and Hanawalt, P. C., *J. Mol. Biol.*, **62**, 65 (1971).
- ¹¹ Fakan, S., Turner, G., Pagana, J., and Hancock, R., *Proc. US Nat. Acad. Sci.*, **69**, 2300 (1972).
- ¹² Kuo, C. H., and August, J. T., *Nature*, **237**, 105 (1972).
- ¹³ Schachtele, C. F., Anderson, D. L., and Rogers, P., *J. Mol. Biol.*, **49**, 255 (1970).
- ¹⁴ Iwaya, M., and Denhardt, D. T., *J. Mol. Biol.*, **57**, 159 (1971).

An Extinct Spreading Centre in the Philippine Sea

Z. BEN-AVRAHAM & C. BOWIN

Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

J. SEGAWA

Ocean Research Institute, University of Tokyo, Nakano, Tokyo

An extinct ocean floor spreading centre identified by a topographic ridge has been discovered in the West Philippine Basin. Evidence of active spreading during the Mesozoic explains the early evolution of the Western Pacific and some marginal seas.

LINEATED magnetic anomalies in the western Pacific have been reported in the western Pacific Basin¹, in the Caroline Basin², and in the China Basin³. The evolution of portions of the western Pacific has been interpreted in terms of the separation and rotation of crustal blocks^{4,5}, but until now no site of crustal accretion had been well documented in the western Pacific.

In a recent cruise of the RV Hakuho Maru of the Ocean Research Institute of the University of Tokyo, a north-south geophysical profile was made in the western Philippine Basin (Fig. 1). This profile crossed an apparently continuous ridge and rift system in the bathymetric map of Chase and Menard⁶. The ridge, which we call the Philippine Ridge, runs 1,500 km from the Palau-Kyushu Ridge towards the southern edge of the Ryukyu Trench along a strike of N 50° W. A Soviet morphological map⁷ also shows this ridge very dramatically.

Early works by Hess⁸ and Dietz⁹ noted the existence of a structural element in this area. Hess⁸ called it a "central basin fault" and suggested that it is part of a master rift zone extending across the entire Philippine Sea. Khain and Muratov¹⁰ followed Hess's idea and called this feature a deep fault.

Bathymetry, magnetic and gravity profiles over this ridge are similar to those of a mid-ocean ridge (Fig. 2). Its width is 500 km, rising from an average depth of 6,200 m in the basin to 4,350 m at its peak. The general topography and dimensions—the rift valley, for example, has a relief of 2 km and width of 25 km—resemble those of the Mid-Atlantic Ridge¹¹, except that its average depth is greater by about 2 km and the relief from edge to crest is reduced. Seismic refraction studies¹² show that the area is underlined

by a normal oceanic crust 5 to 6 km thick. The thickness of the low velocity sediments was found to be less than 100 m. There is no seismic activity at present over the entire Philippine Basin¹³.

The gravity signature of the Philippine Basin is also similar to that of the Mid-Atlantic Ridge¹⁴. The free-air gravity anomalies are very near zero; at the central rift valley a maximum value of +42 mgal (and a maximum peak-to-peak relief of 57 mgal) is observed. Elsewhere the anomalies are generally 0+15 mgal. The anomalies are slightly more positive in the crestal region than on the flanks as is common for active spreading ridges of the world¹⁵. Here, however, a shallow free-air minimum is found about

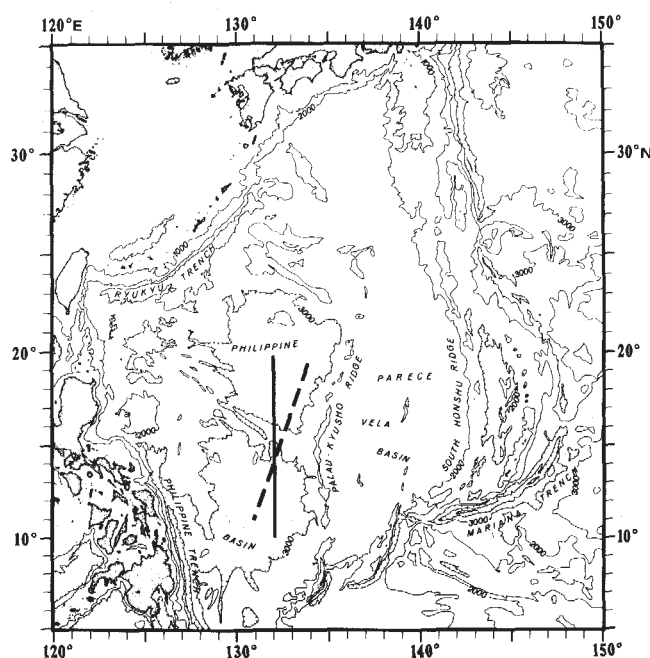


Fig. 1 Simplified bathymetric map of the Philippine Sea, based on Chase and Menard⁶. Contour interval every 1,000 fathom. Heavy line indicates the position of the profile taken by RV Hakuho Maru and heavy-dashed line the position of a profile taken by RV Umitake Maru in 1963.

300 km from the crest on the north side, and about 150 km from the crest on the south side. Both these free-air minima are correlated with depressions in the average depth of the flank topography, and the averaged Bouguer anomaly profile shows no associated perturbation. It may be that the ridge has subsided and in so doing the crestal material has acted as a load and slightly depressed the bordering portions of the crust.

The regional Bouguer anomaly gradient at the crest is $0.37 \text{ mgal km}^{-1}$, diminishing to $0.17 \text{ mgal km}^{-1}$ at about 120 km distance. These values are less than those normally observed at similar distances from the crest of the Mid-Atlantic¹⁶, and probably indicate that the zone of compensation has both deepened and become less steeply dipping away from the crest as the crest has subsided following cessation of spreading.

The most striking feature associated with this ridge is the magnetic anomaly pattern, which displays an almost perfect symmetry about its axis (Fig. 3). The anomalies have average amplitude of 200 km and wavelength of 30–60 km. We have attempted to determine the structure responsible for this magnetic anomaly pattern by constructing a magnetic block model based on the Vine–Matthews hypothesis¹⁷. Such a model cannot explain the observed magnetic anomalies if one uses the present strike of the ridge (N 50° W) and the present field strength and direction, because this configuration will produce asymmetrical magnetic anomalies over a symmetrical two-dimensional magnetization distribution. If, however, the ridge strike is taken to be N 10° W the model yields magnetic anomalies similar to those observed (Fig. 3). An alternative way to generate more symmetric anomalies without changing the strike of the body is to change the latitude of the model closer to the equator (Fig. 3), because east-west striking crustal symmetry will produce symmetrical magnetic anomalies in only the highest and lowest magnetic latitudes¹⁸. In these models we have assumed that the magnetic field at the time of generation of this oceanic crust had the same character as the present field.

We computed simulated magnetic anomalies from the Cainozoic time scale of Heirtzler *et al.*¹⁹ for different spreading rates, azimuths, and latitudes and found no good correlation with the observed symmetric profile. It seems, therefore, that the anomalies are older than anomaly 32 and probably formed in the Mesozoic. We number the observed anomalies as P_1 to P_7 using the letter P for Philippine.

If the crust of the Western Philippine Basin is proved to be

Mesozoic by deep-sea drilling, then this sequence of anomalies will offer a good Mesozoic time scale because they are so well expressed and show such a remarkable symmetry across the ridge.

A comparison of the elevation of the Philippine Ridge with the empirical depth/age plots of Sclater *et al.*²⁰ suggests an age of about 58 m.y. for the ridge crest. This assumes, following Sclater *et al.*, that the elevation at the ridge crest, a fixed time after spreading terminated, is the same as the elevation it would have reached if it had been spreading for the same amount of time and that this is true for all spreading rates. The depths of the flanks of the Philippine Ridge (6,200 m) are greater than those encountered by Sclater *et al.*²⁰ and thus most likely the 58 m.y. age is a minimum age.

The existence of the deep rift valley and high rift mountains in this extinct spreading centre is very pertinent to the origin of crestal topography and the evolution of flank topography with age. The rift valley topography of the Philippine Ridge is among those with the most extreme relief. The depth of the rift valley actually exceeds the depth of the flanks. The original local relief appears to have been retained for more than 60 m.y. while the ridge as a whole underwent subsidence. The rough topographic expression of the ridge and the pronounced central rift suggest that the spreading rate was less than 2.5 cm yr^{-1} (refs. 16, 21).

Two other shipboard and one airborne magnetic anomaly profiles are known across this ridge²²: Umitake Maru, 1963; Hakuho Maru, 1971; and Project Magnet flight 311. In the airborne record near longitude 127° E, being taken at a higher altitude, fewer anomalies are observed, but they are consistent with a symmetrical pattern. One of the other shipboard profiles is shown in Fig. 3. The shipboard data demonstrate rather good correlation with the anomalies found in cruise Hakuho Maru, 1972, and the continuity of the ridge as a spreading centre between 129° E and 132° E longitudes.

The discovery of an extinct spreading ridge in the Philippine Basin is of great importance in understanding the tectonic evolution of the whole western Pacific and in particular the marginal seas. Because this is the first and so far the only spreading centre to be identified in the Western Pacific, we feel that the magnetic lineations reported in the western Pacific previously should be re-examined to check their relation to the spreading ridge. This ridge also offers an attractive alternative to the recent proposals for the evolu-

Fig. 2 Bathymetry magnetics and gravity profile taken by RV Hakuho Maru. The position of the profile shown in Fig. 1. *a*, Bouguer; *b*, free air; *c*, magnetics; *d*, bathymetry.

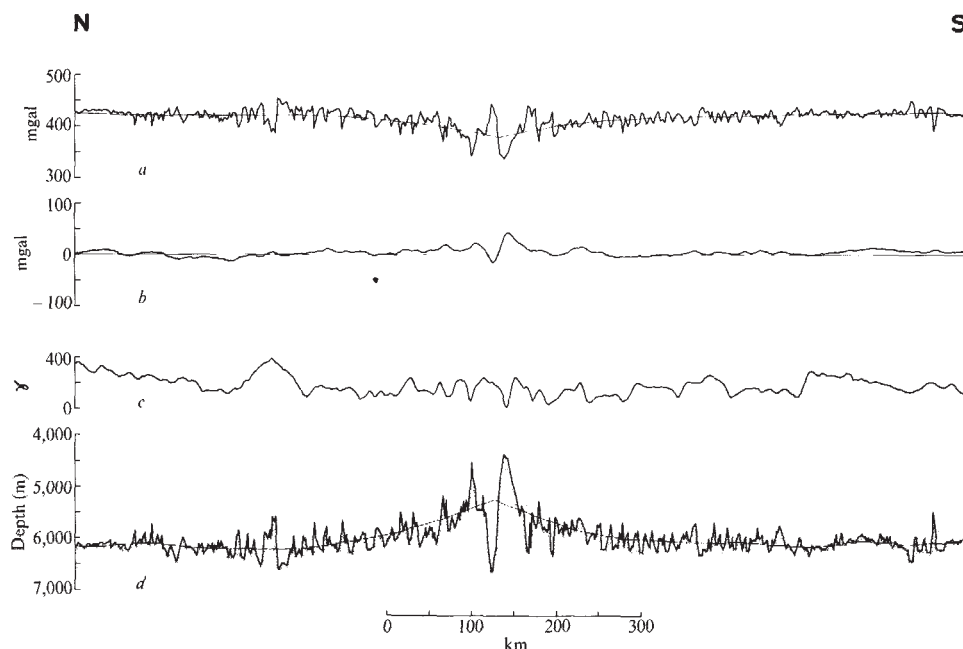
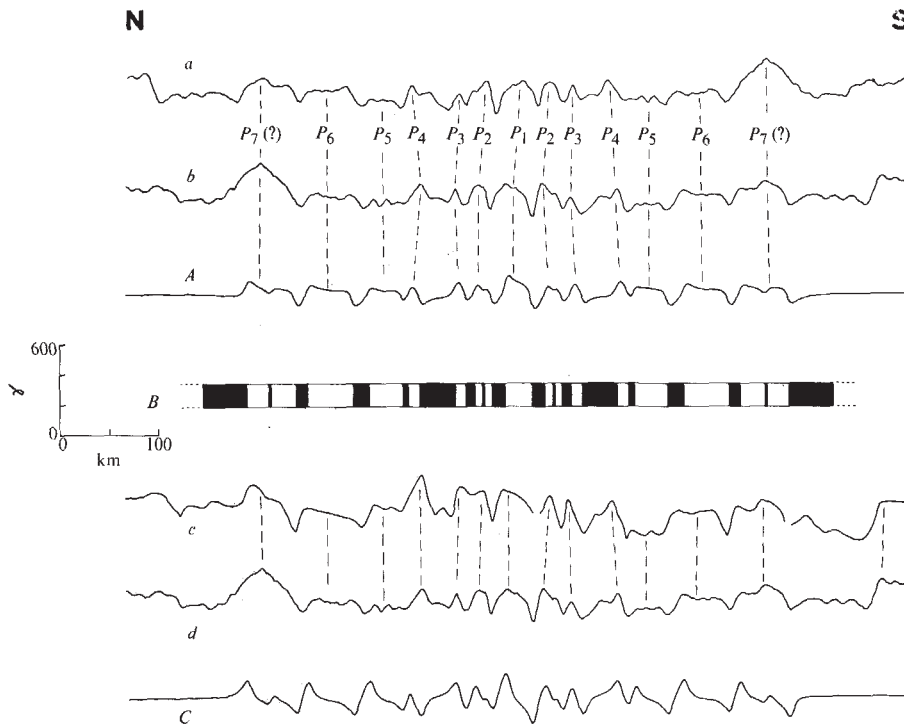


Fig. 3 Magnetic profiles: observed and simulated. North is on the left and south on the right except for the reversed profile. *a* and *b*, Hakuho Maru data, 1972, of observed magnetic anomaly profiles, whose positions are shown in Fig. 1. Also shown, a simulated profile (A) generated by sea-floor spreading model B. The model can be generated either by a ridge striking N 10° W at latitude 15° N or N 50° W at latitude 8° N. The susceptibility for the strike of N 10° W taken to be 0.02 c.g.s. units and for the strike of N 50° W is 0.01 c.g.s. units. The polarity of the blocks is opposite for the two models. Black indicates normal magnetized blocks for a ridge striking N 10° W while for a ridge striking N 50° W white indicates normal magnetized blocks. The model assumes a magnetized layer at a depth of 5.5 to 7 km. The total field intensity was taken to be 42,000 gammas. *c*, Data from Umitaka Maru (1963) and *d*, data from Hakuho Maru (1972), providing two observed magnetic anomaly profiles, whose positions are shown in Fig. 1. Also shown a simulated profile (c) from a ridge striking N 50° W at 15° N which are the present strike and latitude of the ridge in the Philippine Basin. Other parameters are the same as for the simulated profile at the top. Note that the model produces asymmetrical anomalies.



tion of marginal seas. Karig²³ suggests that these basins are formed by crustal extension within an old arc which forces the trench to migrate seaward and further that the west Philippine Basin formed in a similar way²⁴. Finding this extinct ridge invalidates that explanation of the west Philippine Basin.

The block model of Fig. 3 gives two possibilities as to the original position of the ridge; either at the same geographic latitude as at present but at different orientation, or at the same orientation but at different latitude. The first requires a counterclockwise rotation of the western Philippine basin by about 40° while the second requires a northward movement of the basin by about 800 km. We favour the second possibility for two reasons. First, the major fault along the Philippine Islands is left lateral and does not fit the sense of motion required to rotate the western Philippine Basin from N 10° W to N 50° W; it does fit a northward motion of this entire block. Second, all the northward motion could be absorbed by the Ryukyu Trench which already existed in the Mesozoic²⁵ and by the remnant arcs Oki Daito and Daito²⁶ trending parallel to the Philippine Ridge about 1,000 km to the north. While the Mesozoic evolution of the west Philippine Basin involved the generation of an oceanic crust from a northwest-southeast trending mid-ocean ridge perhaps by the same mechanisms which operate in the major ocean basins, the Tertiary evolution of the Philippine Sea seems to be different. The Tertiary evaluation involved the formation of north-south trending elements: the Palau-Kyushu Ridge, the Parece Vela Basin, and the Mariana Island Arc system which may have a different mechanism of formation²⁴.

We offer that at least some of the marginal seas are the result of sea floor spreading centred on oceanic ridges. These ridges may be confined to the basins themselves, or may be, as we believe, part of larger ridge systems extending into the Pacific Basin. We suggest that the Philippine Basin was once part of the Northwest Pacific Basin but is now separated from it by the formation in the Tertiary of the Palau-Kyushu Ridge, the Parece Vela Basin and the Mariana Island Arc system.

We thank the Captain, Ichiro Tadama; the Chief Scientist, Ryuzo Marumo; the officers and the crew of the RV Hakuho Maru for their help, and the US Army Topographic

Production Center for the loan of Equipment. This work was supported by the US Office of Naval Research and by the National Science Foundation. This study is part of a US/Japan cooperative science programme. We thank J. R. Heirtzler, J. D. Phillips and E. T. Bunce for discussions and criticisms.

Received September 25, 1972.

- ¹ Larson, R. L., and Chase, C. G., *Trans. Amer. Geophys. Un. (Abstr.)*, **53**, 532 (1972).
- ² Lowrie, A., Bracey, D. R., and Vogt, P. R., *Amer. Geophys. Un. (Abstr.)*, **53**, 413 (1972).
- ³ Ben-Avraham, Z., and Phillips, J. D., *Amer. Geophys. Un. (Abstr.)*, **53**, 519 (1972).
- ⁴ Milson, J. S., *J. Geophys. Res.*, **75**, 7335 (1970).
- ⁵ van der Linden, W. J. M., *Earth Planet. Sci. Lett.*, **6**, 483 (1969).
- ⁶ Chase, T. E., and Menard, H. W., *Bathymetric Atlas of the Northwest Pacific Ocean* (1969).
- ⁷ Udintsev, G. B., *Tixi okean, Akad. Nauk. SSSR, P. P. Shirshov*, 394 (Institute Okeanologii, Moscow, 1972).
- ⁸ Hess, H. H., *Geol. Soc. Amer. Bull.*, **59**, 417 (1948).
- ⁹ Dietz, R. S., *Geol. Soc. Amer. Bull.*, **65**, 1199 (1954).
- ¹⁰ Khain, V. E., and Muratov, M. V., *The Earth's Crust and Upper Mantle* (edit. by Hart, P. J.); *Geophys. Mono.*, **13**, 523 (Amer. Geophys. Un.); (1969).
- ¹¹ Heezen, B. C., Tharp, M., and Ewing, M., *Geol. Soc. Amer. Special Paper*, **65** (1959).
- ¹² Murauchi, S., Den, N., Asano, S., Hotta, H., Yoshii, T., Asanuma, T., Hagiwara, K., Ichikawa, K., Sato, T., Ludwig, W. J., Ewing, J. I., Edgar, N. T., and Houtz, R. E., *J. Geophys. Res.*, **73**, 3143 (1968).
- ¹³ Barazangi, M., and Dorman, J., *Seism. Soc. Amer. Bull.*, **59**, 369 (1969).
- ¹⁴ Talwani, M., LePichon, X., and Ewing, M., *J. Geophys. Res.*, **70**, 341 (1965).
- ¹⁵ Talwani, M., *The Sea* (edit. by Maxwell, A. E.), **4**, 251 (1970).
- ¹⁶ van Andel, T. H., and Bowin, C. O., *J. Geophys. Res.*, **73**, 1279 (1968).
- ¹⁷ Vine, F. J., and Matthews, D. H., *Nature*, **199**, 947 (1963).
- ¹⁸ Schouten, J. A., *Marine Geophys. Res.*, **1**, 111 (1971).
- ¹⁹ Heirtzler, J. R., Dickson, G. O., Herron, E. M., Pittman, W. C., III, and LePichon, X., *J. Geophys. Res.*, **73**, 2119 (1968).
- ²⁰ Sclater, J. G., Anderson, R. N., and Bell, M. L., *J. Geophys. Res.*, **76**, 7888 (1971).
- ²¹ Menard, H. W., *Science*, **157**, 923 (1967).
- ²² Tomoda, Y., Ozawa, K., and Segawa, J., *Bull. Ocean Res. Inst., Univ. Tokyo*, **3**, 169 (1968).
- ²³ Karig, D. E., *J. Geophys. Res.*, **76**, 2542 (1971).
- ²⁴ Karig, D. E., *Geol. Soc. Amer. Bull.*, **82**, 323 (1971).
- ²⁵ Meng, C. Y., *Abstract, Twelfth Pacific Sci. Congress*, **1**, 377 (1971).
- ²⁶ Karig, D. E., *Geol. Soc. Amer. Bull.*, **83**, 1057 (1972).

LETTERS TO NATURE

PHYSICAL SCIENCES

On a Possible Interstellar Galactic Chromosphere

I SUGGEST here that the disk of the Galaxy is surrounded by a shell of gas at a temperature intermediate between the 10^2 – 10^4 K of the disk and the 10^6 K of the corona¹, and that this "chromosphere" may be detectable. This suggestion is related to the hypothesis^{2,3} that the Galaxy is accreting hot gas from the intergalactic space of the Local Group. According to the calculations of Hunt⁴, if accretion is occurring the rate of mass influx $\sim 2n_{-4}/T_6 M_\odot$ per year, where n_{-4} and T_6 are the unenhanced density and temperature of the intergalactic gas in units of 10^{-4} atoms cm^{-3} and millions of degrees respectively, and the mass and velocity of the Galaxy are taken to be $1.7 \times 10^{11} M_\odot$ and less than $\sim 100 \text{ km s}^{-1}$. We shall adopt as representative values $n_{-4} \sim 3$ and $T_6 \sim 2$ (for further discussion and references, see ref. 3) which lead to an accretion rate $\sim 3 M_\odot$ per year. This rate is compatible with dynamical considerations concerning the motion of interstellar gas in the disk of the Galaxy, and with the age of the Galaxy, the present density of this gas and estimates⁵ of the rate of star formation.

This accretion hypothesis has thermal consequences as well as dynamical ones. Over most of the flow cooling would be unimportant³, but eventually the gas must lose its heat as it approaches the relatively cool disk of the Galaxy. It seems likely that the decisive factor is the increasing abundance of elements heavier than helium as the disk is approached, because radiative cooling by these elements would be more important than by hydrogen and helium even if their abundances relative to hydrogen were only 10% of the disk values. There are two problems in making a detailed calculation of this cooling process. First, we know only roughly how the heavy element abundances vary with height above the galactic plane. Second, it turns out that the ionization stages of the heavy elements are not mainly determined by collisions (as they are in the solar chromosphere), but by photo-ionization due to the radiation emitted in the cooling layer itself. The resulting transfer problem is a complex one, which would require extensive numerical work even if the heavy element abundances were known (cf. refs. 6, 7).

We content ourselves here with rough estimates. With our adopted parameters the heat influx into each face of the galactic disk $\sim 3 \times 10^{-5} \text{ erg cm}^{-2} \text{ s}^{-1}$. We shall assume that there is available for radiating this heat a heavy element abundance relative to hydrogen $\sim 10\%$ of the disk value. The ionization equilibrium is unknown, but the total radiation rate is likely to be of a similar order of magnitude to that associated with collisional ionization equilibrium in the temperature range $2 \times 10^6 \text{ K}$ – $2 \times 10^4 \text{ K}$. According to Cox and Tucker⁸, the rate varies over this temperature range by only a factor of 20, with an average value (for our adopted abundance) $\sim 3 \times 10^{-23} n_e^2 \text{ ergs cm}^{-3} \text{ s}^{-1}$, where n_e is the electron density in cm^{-3} in the emitting region. Adopting this average value for the radiation rate we find that an emission measure $\sim 0.3 \text{ cm}^{-6} \text{ pc}$ would be required to radiate away all the incoming heat flux. If the 10% abundance level is attained at a height above the galactic plane $\sim 1 \text{ kpc}$ where $n_e \sim 2 \times 10^{-2} \text{ cm}^{-3}$ (refs. 9 and 10), a layer thickness $\sim 750 \text{ pc}$ would be required. There is presum-

ably then no difficulty in principle about dissipating the heat flux; the problem is rather to compute the temperature profile in the transition layer and the spectrum of the resulting radiation.

We have still to examine whether the incoming gas will have time to cool before it reaches the disk. With our parameters

$$\text{the cooling-time} \sim \frac{1.5 \times 10^6}{n_e} \text{ yr or } 7.5 \times 10^7 \text{ yr for } n_e \sim 2 \times 10^{-2}.$$

The average inflow velocity where the particle density $\sim 2 \times 10^{-2} \text{ cm}^{-3}$ (for a mass accretion rate $\sim 3 M_\odot$ per year) $\sim 10 \text{ km s}^{-1}$, so that a 750 pc layer would be traversed in $\sim 7.5 \times 10^7 \text{ yr}$. Thus there is just sufficient time for the cooling to occur above the disk.

Finally we must consider whether the radiative flux from the galactic chromosphere would have observable consequences. This flux would consist predominantly of line radiation from heavy elements in various stages of ionization. These lines would be distributed throughout the wavelength range from, say, 20 Å to 1000 Å (the various ionization stages of oxygen alone give rise to lines spanning this range). Unfortunately, until the relative abundances of the elements at a height $\sim 1 \text{ kpc}$ are known and the transfer problem solved, we can say very little about the resulting spectrum as it would appear to existing X-ray detectors, that is, ones unable to resolve individual lines. However, by the standards of X-ray astronomy the radiative flux that enters the galactic disk ($\sim 1.5 \times 10^{-5} \text{ erg cm}^{-2} \text{ s}^{-1}$) is appreciable. For instance, according to Gorenstein and Tucker¹¹, the observed flux at the galactic pole between 44 Å and 80 Å (280–160 eV) $\sim 10^{-7} \text{ erg cm}^{-2} \text{ s}^{-1}$. Even allowing for substantial galactic absorption at these wavelengths we see that our estimated flux may make a substantial contribution to the present broadband observations at soft wavelengths. In the future with improved wavelength resolution it should be possible to detect individual lines. If our estimated flux were shared out equally by, say, 150 lines, there would still be $\sim 10^{-7} \text{ erg cm}^{-2} \text{ s}^{-1}$ in each line, which at suitable wavelengths might represent a detectable flux. In addition one might be able to detect far ultraviolet lines, such as He II 1640 Å, CIV 1548, 1551 Å, NV 1238, 1243 Å or OVI 1032, 1038 Å which are beyond the Lyman limit and so would not be strongly absorbed by hydrogen in the galactic disk. These lines might also be observed in absorption in the spectrum of an early type star situated high above the galactic disk. Such lines, in both emission and absorption, should be searched for by ultraviolet satellites. They may also be detectable in emission in the chromospheres of other galaxies such as M31 and M87, if these galaxies are also accreting hot intergalactic gas at a significant rate¹².

The proposed radiative flux of $\sim 1.5 \times 10^{-5} \text{ erg cm}^{-2} \text{ s}^{-1}$ may also have important ionization effects in the Galaxy. Following the discussion of Felten and Bergeron¹³ and of Silk¹⁴ we would expect a partially ionized slab to be formed just outside the galactic disk. Silk identified this slab with that proposed by Purton⁹ and by Bridle and Venugopal¹⁰ to account for the absorption of 10 MHz radiation and the dispersion measure of high latitude pulsars. To obtain this result, however, Silk had to use an ionizing flux of unspecified origin ~ 10 times greater than had been observed when he wrote his paper. Here we would tentatively attribute the ionizing flux to soft X-rays which are produced in the galactic

chromosphere and which ultimately derive their energy from the accretion of hot gas by the Galaxy. Whether the flux of ionizing photons is sufficient depends on its unknown spectrum, but in principle there would be enough flux available.

A further possibility is that sufficient photons may enter the disk of the Galaxy to contribute appreciably to the overall ionization rate of the interstellar medium. We know from the work of Bergeron and Souffrin¹⁵ and of Habing and Goldsmith¹⁶ that if the disk is ionized by ~ 100 eV photons then we would need a primary hydrogen ionization rate $\sim 10^{-16} \text{ s}^{-1}$ to account for the observed ionization state and temperature of the interstellar medium. The actual ionization mechanism is still not known. A likely possibility is radiation from the discrete galactic soft X-ray sources discussed by Gorenstein and Tucker¹¹ and by Davidsen *et al.*¹⁷. However, radiation from the galactic chromosphere is an alternative possibility which might be important close to the edge of the disk.

As a final speculative remark I would mention the possibility that it is the inflowing material which is responsible for the confinement of the interstellar magnetic field and cosmic rays to the disk of the Galaxy. This is dynamically reasonable since with our accretion parameters the internal and ram pressures of the incoming material $\sim 10^{-12} \text{ dyne cm}^{-2}$ near the disk. I hope to return to this dynamical question elsewhere.

I thank R. Hunt, N. L. Balazs, A. Gabriel and M. S. Longair for helpful discussions and M. J. Rees for valuable criticisms of the first version of this letter.

D. W. SCIAMMA

Department of Astrophysics,
Oxford University

Received August 17; revised November 7, 1972.

- ¹ Spitzer, L., *Astrophys. J.*, **124**, 20 (1956).
- ² Sciamma, D. W., *Mon. Not. Roy. Astron. Soc.*, **123**, 317 (1962).
- ³ Hunt, R., and Sciamma, D. W., *Mon. Not. Roy. Astron. Soc.*, **157**, 335 (1972).
- ⁴ Hunt, R., *Mon. Not. Roy. Astron. Soc.*, **154**, 141 (1971).
- ⁵ Salpeter, E. E., *Interstellar Gas Dynamics*, IAU Symposium No. 39, 221 (Reidel, Dordrecht, 1970).
- ⁶ Tarter, C. B., and Salpeter, E. E., *Astrophys. J.*, **156**, 943 (1969).
- ⁷ Tarter, C. B., Tucker, W. H., and Salpeter, E. E., *Astrophys. J.*, **156**, 953 (1969).
- ⁸ Cox, D. P., and Tucker, W. H., *Astrophys. J.*, **157**, 1157 (1969).
- ⁹ Purton, C. R., thesis, University of Cambridge (1966).
- ¹⁰ Bridle, A. H., and Venugopal, V. R., *Nature*, **224**, 545 (1969).
- ¹¹ Gorenstein, P., and Tucker, W. H., *Astrophys. J.*, **176**, 333 (1972).
- ¹² Hunt, R., and Sciamma, D. W., *Nature*, **238**, 320 (1972).
- ¹³ Felten, J. E., and Bergeron, J., *Astrophys. Lett.*, **4**, 155 (1969).
- ¹⁴ Silk, J., *Astron. Astrophys.*, **12**, 421 (1971).
- ¹⁵ Bergeron, J., and Souffrin, S., *Astron. Astrophys.*, **11**, 40 (1971).
- ¹⁶ Habing, H. J., and Goldsmith, D. W., *Astrophys. J.*, **166**, 525 (1971).
- ¹⁷ Davidsen, A., Shulman, S., Fritz, G., Meekins, J. F., Henry, R. C., and Friedman, H., *Astrophys. J.* (in the press).

Is There a Tenth Planet in the Solar System?

We have been analysing the longitudinal residuals of Neptune (N), partly from sheer interest aroused by the discordance between the pre-discovery position of N and its currently accepted orbit¹ and because colleagues (already conducting a photographic hunt for a hypothetical tenth planet) sought our assistance in narrowing the field of search by using perturbation theory.

Seidelmann² has ruled out any of three possible outer planets—and Brady's suggestion³ of a planet of Jovian mass at about 60 AU seems hardly possible, because its effect on Uranus (U) and N (easily computed from ref. 4, Fig. 4) would be larger by orders of magnitude than any residual observed since the mid nineteenth century. Further, a search by Foss *et al.* has failed to find it⁵. We therefore decided to attempt to

establish the limits of the possible position of a tenth planet, working from data on N.

N was chosen as the main basis for the work because it is the most sensitive of the known planets to the pull of a possible exterior body⁴ and the least affected by existing uncertainties in the masses of its known planetary neighbours. As a check on the working, data from Uranus (U) were also used; and cases where the results from these two sources were markedly different were rejected.

A least-squares differential analysis was carried out, assuming circular orbits for any perturbing body. This assumption was made because it is reasonable *a priori*, and because it was decided that the N data were insufficient to indicate reliably the extra unknowns involved in an elliptical path. (A paper giving full details of the work is in preparation.)

The only satisfactory solutions found were for bodies whose orbits lay between 50 and 100 AU from the Sun, and whose positions at 1973.0 lay within a relatively small sector of the ecliptic. The range of such possible bodies is shown in Table 1.

Table 1 Limits of Solutions for a Possible Tenth Planet

Radius of orbit (AU)	Longitude limits (2 s.d.)	Mass ± 1 s.d.
75	309°–320°	5 ± 2
60	322°–343°	3 ± 1
50	338°–5°	2 ± 1

Masses are in Sun/206265.
(Epoch: 1970.0; E and E 1950.0.)

These results and N's latitude residuals were used to find latitude solutions. Unfortunately, however, the standard deviations of the latitude results are large, and the confidence intervals correspondingly so (Table 2).

Table 2 Latitude Solutions for Various Radius and Longitude Values

Radius of orbit (AU)	Longitude	Latitude ± 1 s.d.
75	310°	$-17^\circ \pm 37^\circ$
	320°	$-27^\circ \pm 40^\circ$
60	320°	$+6^\circ \pm 8^\circ$
	340°	$+30^\circ \pm 17^\circ$
50	350°	$+14^\circ \pm 6^\circ$
	0°	$+20^\circ \pm 10^\circ$

Seidelmann² rejected 3 trial planets, as the only one which accounted for the 1795 residual forced unacceptably large ones upon N's motion since 1846. A body falling within our range, however, satisfies the former whilst only requiring disturbances of amplitude ca. 0.05 s in the post-1846 data.

Nevertheless we do not definitely assert that such a planet exists. Our set task, which we consider we have performed, was to delimit the possible space wherein it might be.

If the current search in this area finds nothing, then the indication becomes strong that the major bodies of the solar system end at N, a matter bearing critically upon theories of the origin of the solar system.

We thank Dr D. Barton, Dr J. Rozics, Mr J. Lang, Miss E. Sadie and Miss A. Avirett for their assistance in the work and also Dr D. W. Dewhirst for help and advice.

D. RAWLINS

College of Notre Dame of Maryland, Baltimore

M. HAMMERTON

APU Cambridge, England

Received October 17, 1972.

- ¹ Rawlins, D., *Astrophys. J.*, **75**, 856 (1970).
- ² Seidelmann, P., *Astrophys. J.*, **76**, 740 (1971).
- ³ Brady, J., *Publ. Astr. Soc. Pacific*, **84**, 314 (1972).
- ⁴ Rawlins, D., *Mon. Not. Roy. Astron. Soc.*, **147**, 177 (1970).
- ⁵ Foss, A., Shawe-Taylor, J., and Whitworth, D., *Nature*, **266**, 239 (1972).

Are the Lunar Seismic Signals compatible with a Deep Layer of Fine Powder?

THE seismic wave velocity may vary with depth in the Moon in the way derived from the lunar seismic signals by Toksöz *et al.*¹ It is customary to seek sudden changes in velocity and interpret these as evidence for sudden changes in mineral type. Here it is shown that one such sudden change at 25 km and the more gradual increase in velocity from the surface to it can be interpreted in terms of a single type of material, namely the fine rock powder that is so abundant on the lunar surface. Gold and Soter² have shown how such a deep powder layer can give rise to the general features of the lunar seismic signals, which are clearly so different from those seen in the Earth.

Good quantitative agreement has been obtained between lunar near surface velocities and values obtained in rock powders in the laboratory³. However, Toksöz *et al.*¹ rule out a deep powder layer. They derive a velocity profile for powders from laboratory data on lunar fines⁴ by using values of velocity corresponding to the maximum pressure to which the fines were exposed in the laboratory. They thus obtain the result, which we shall see is not necessarily correct, that the depth dependency of velocity in lunar fines would be slower than the actual depth dependency inferred from the seismic signals.

The densification process accompanying an increase in velocity in powders is rate controlled by pressure, and by some diffusion constant which is temperature sensitive⁵. The process is thus non-stationary, until full compaction is reached. In the Moon the powder will have been compressed for times 10^{11} longer than in laboratory studies and therefore densification there has occurred to a far greater extent. This will be all the more so the greater the depth and the greater the temperature, because the non-stationary processes become more important at greater pressures and temperatures. If, as seems likely, the temperature in the Moon increases with depth then a particularly sharp divergence from laboratory results will occur, though the pressure increase with depth alone is also significant. Therefore the rate of increase of velocity with pressure observed in the laboratory is only a lower limit to the inferred rate of increase with depth in the Moon.

The laboratory data⁴ show that the memory of earlier periods of higher pressure is preserved at later lower pressures in the form of velocities that exceed those in powders that have only been compressed to the same lower pressure. The relevant pressure to the densification rate is therefore some pressure that is between the current pressure and the maximum pressure. Material raining down on the Moon is a plausible source of earlier higher pressure and this possibility has to be taken into account when extrapolating laboratory data to lunar circumstances.

Between 23.5 and 26.5 km the velocity appears to increase by a comparatively large amount; by 13% from 5.8 to 6.8 km s⁻¹, the latter figure being typical of low porosity crack free rocks. Thereafter the velocity increases by only about 1% down to 65 km. The comparatively sudden increase of 13% can be explained in terms of final densification in fine rock powder.

The experiments of Rossi and Fulrath⁵ show that during the final stages of densification in alumina the porosity Γ goes as

$$\Gamma \sim \Gamma_1 \exp \left\{ -3.4 \times 10^{-5} \frac{PDt}{(\bar{l}_g)^2 T} \right\} \quad (1)$$

in c.g.s. units, where P is the confining pressure, D is a diffusion constant, t is time, $\bar{l}_g$ is mean grain size, T is in K and Γ_1 is a porosity above which final densification processes are the dominant ones. For alumina the slope of $\log_{10} D$ versus $1/T$ gives an activation energy of 115 kcal mol⁻¹, which is typical of diffusion processes in many materials. The value of D at any one temperature, however, varies by orders of magnitude

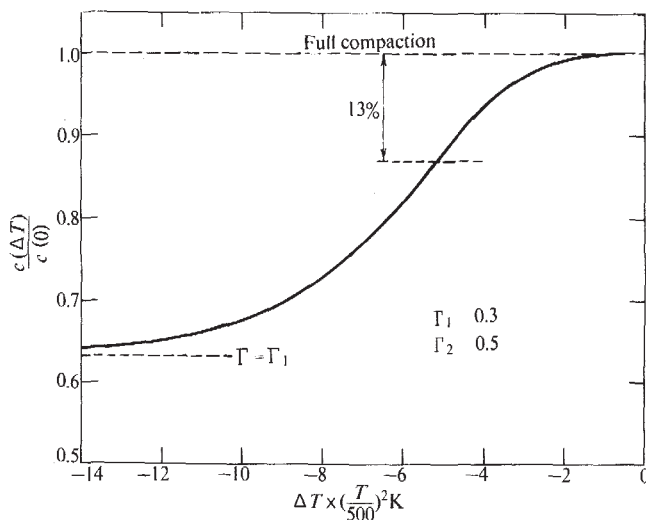


Fig. 1 A sharp increase in velocity c near full compaction in a fine rock powder, such as may occur near 25 km in the Moon. $\Gamma_1 = 0.3$; $\Gamma_2 = 0.5$.

between different materials and is also sensitive in any one material to the density of various lattice defects.

At 25 km in the Moon $P \sim 1.3 \times 10^9$ dynes cm⁻², so with $t \sim 1.3 \times 10^{17}$ s ($\sim 4 \times 10^9$ yr), $\bar{l}_g \sim 10^{-4}$ cm, $T \sim 850$ K, $D \sim 8.5 \times 10^{-27}$ cm² s⁻¹, from equation (1) we obtain $\Gamma \sim 0.003\Gamma_1$, which is essentially full compaction. If 850 K is a higher effective temperature than would be expected at 25 km from the history of the lunar temperature profile then there is plenty of freedom to raise D and lower $\bar{l}_g$ in order to lower this temperature.

Full compaction at 25 km is thus feasible. Below this depth the fine powder would be a low porosity crack free rock, with corresponding velocities.

To obtain an increase of 13% in velocity c requires that the mean contact radius $\bar{r}_c$ increases by about 28%. This is seen from the easily established relation, $c \propto \sqrt{\bar{r}_c}$. Elementary geometrical considerations show that $\bar{r}_c \propto (\Gamma_2 - \Gamma)$ approximately, where Γ_2 is a porosity above which plastic deformation at contact points is giving rise to appreciable values of $\bar{r}_c$. Certainly $\Gamma_2 > \Gamma_1$. Values of $\Gamma_2 \sim 0.5$ and $\Gamma_1 \sim 0.3$ yield Fig. 1 for c versus temperature.

The 13% increase in velocity over 3 km thus only requires that the temperatures at two places 3 km apart in depth near 25 km in the Moon have been a few degrees different for an appreciable part of the Moon's lifetime. This result is not very sensitive to the values adopted for Γ_1 and Γ_2 .

Other steps in the velocity depth curve may occur, but within the first few hundred metres of the surface. These would arise when the particle adhesion stresses of a few hundred dynes cm⁻² are being overcome⁶, and at greater depth in the region of the onset of particle fracture. There are as yet insufficient data to decide whether such steps have been seen in the Moon.

I thank Professor T. Gold and Dr Malcolm J. Campbell for valuable discussions. This work was supported by a NASA grant.

B. W. JONES

The Open University,
Walton Hall,
Bletchley,
Buckinghamshire

Received August 27, 1972.

¹ Toksöz, M. N., Press, F., Anderson, K., Dainty, A., Latham, G., Ewing, M., Dorman, J., Lamlein, D., Sutton, G., Duennember, F., and Nakamura, Y., *Science*, **176**, 1012 (1972).

² Gold, T., and Soter, S., *Science*, **169**, 1071 (1970).

³ Jones, B. W., *Proc. Third Lunar Sci. Conf.*, 3 (in the press).

- ⁴ Warren, N., Schreiber, E., Scholz, C., Morrison, J. A., Norton, P. R., Kumazawa, M., and Anderson, O. L., *Proc. Second Lunar Sci. Conf.*, 3, 2345 (1971).
⁵ Rossi, R. C., and Fulrath, F. M., *J. Amer. Cer. Soc.*, 48, 558 (1965).
⁶ Selheimer, H. E., and Johnson, S. W., *J. Geophys. Res.*, 74, 5321 (1969).

The Solution to Professor Runcorn's Problem

AT the meeting of the Royal Astronomical Society on January 9, 1970, I drew attention to some mathematical and numerical errors in a theoretical discussion by S. K. Runcorn of his proposed mode of development of an iron-core for the Earth¹ which he regards as providing strong support for his hypothesis of continental drift. Runcorn has recently defended his theory², but the proffered justifications involve serious confusion of the dubiously known physical properties of the actual Earth with the completely known details of the hypothetical problem that he has enunciated and must have in some way precisely defined in order to make it the subject of machine calculations. It is against the validity and correctness of the mathematical steps that he claims to have made that my criticisms have been lodged. The points at issue are not controversial but simply a question of whether or not mathematical errors have been made, matters upon which there can ultimately be no serious ground for sustained disagreement.

Runcorn claims to have found a linear series of Earth models by means of inward integration starting always from a unique surface-radius, the same for each and every model, making use of one particular pressure-density relation and an assumed surface-density at first, and then switching to a different relation at the boundary of the iron core $r=r_c$. This is stated to have been done by a process of trial and error, and to determine at what value for r_c to make the switch Runcorn explicitly claims to have used the requirements that gravity and the pressure-gradient must both vanish at the centre. My contention is that any solution of the relevant equations will automatically satisfy these conditions, and that they therefore provide no criterion at all for finding r_c . It follows that an infinity of solutions satisfying his stated conditions would emerge for each assumed surface-density, and hence in all that there would be a double infinity of values of the gravitational energy, corresponding to a single infinity of surface density values or of core-mass values. How these have been compressed into a single curve of W (the energy) against r_c , or against time, is not discoverable from Runcorn's original paper nor from his recent reply².

This in my view is the principal error of his treatment, one that invalidates his entire discussion, but now there are others in his subsequent claims. However, as it would take much of your valuable space to deal with them all, perhaps I could be allowed, for those of your readers not completely familiar with the theory of planetary structure, to illustrate by means of a homely parable the nature of the confusions in Professor Runcorn's letter between the theoretical procedures, on the one hand, and the actual system to which (it is his hope) they may in some degree relate, on the other.

Let it be imagined then that Surveyor Runcorn is moved to determine the acreage of Farmer Giles's fields with a view to finding how much cash (energy) might be extracted from the tenant. For this purpose our surveyor decrees that each field will be regarded as theoretically representable by a rectangle of sides a and b , whose values when it comes to the arithmetic will be taken from some early results found by Squire Bullyhard (though in fact some of these refer to quite different fields, but never mind that trifle). Proceeding on, Surveyor Runcorn next sets up an impressive mathematical formalism according to which $A = \text{area of field} = \text{area of rectangle} = \pi(a + b + q + r)^2$, wherein q is a certain length associated with each field and is stated to have been chosen by an elaborate process to ensure

first that all the angles are right angles and second that opposite sides are equal, while r is a certain length, the same for every field. By means of this formula, but without disclosing exactly how, he duly produces a series of acreages for the fields. These are published in non-detailed form along with an inadequately brief account of his method. All remains quiet for a time, but then along comes some local yokel (Lyttleton by name) who, having read the surveyor's own account of the matter in the rural news, points out that there is a well-understood theory of how to handle rectangles, which is not that used by a long chalk, and, what is more, the areas given for some of the fields are bigger than that of the whole farm! And he asks rhetorically, "What sort of surveying do you call this?". After some delay, our professional surveyor meets the difficulty, unabashed, by saying that he merely made an error of a decimal point, which could not possibly have misled anyone (apart from meanwhile having upped Giles's rates a tidy bit), and adding that fields are well known to have ditches and hedges, are not perfect rectangles, and do not have accurately straight boundaries; it is therefore quite absurd for that porcher (R. A. L.) to think that the area of a field can be computed accurately anyway. And as if this were not answer enough, the surveyor also claims that these particular fields give a very poor crop, and that there is such a bundle to be forked out annually on the mortgage that as far as the farmer is concerned the acreages, if they could be properly calculated in the light of full knowledge of economic theory, might very well turn out to be negative. So there. But the critic happens to know a little about surveying himself, and insists that there are theoretical ways of working out the area of a rectangle quite accurately when the sides are given, without querying whether the figures adopted for them by the surveyor himself are right. He also has indisputable information that the farm has an excellent yield, and that Farmer Giles owes not any man. And besides, what about that alleged formula for the area of a rectangle? And even supposing it were right, how about explaining how that quaint quantity q can be settled, when it is a property of rectangles that its angles are right angles and its opposite sides equal? And why should r be the same for each and every field? These are all points our *soi-disant* surveyor keeps noticeably quiet about.

This illustrates the kind of state things have been brought to by Professor Runcorn's letter, and it seems less than likely that he will be influenced by any repetition of my arguments if he has not been able to admit the force of them already. Professor Runcorn considers my ideas about mountain-building to be twenty-five years out of date, but the mathematical theory that he flouts has been well understood for over a century, and if and when he can be induced to apply it properly to his problem he may well find that instead I am only minus twenty-five years behind him. Furthermore, the matter need by no means be left open as yet another essentially irresolvable conflict or mystery, like that of Edwin Drood, for there exists a quite simple means whereby Professor Runcorn can resolve the points at issue for us all and completely vindicate his stand into the bargain. And this is merely by producing his original computer program, including the starting-values, and specifying which particular table of the many given by Bullard was made use of for the pressure-density relations and exactly how. With this devastating weapon lying to hand in his armoury, it says a great deal for Professor Runcorn's careful restraint that he has so far neglected to use it to annihilate his critic, but now I beseech him to do so; to point it at my head, and fire. If he will supply this program in any recognized computer-language, it will be scrutinized and processed and re-run, with complete impartiality, to produce all numerical values relevant to the problem, if this is logically possible. With this accomplished, the results or outcome can then be published to enable everybody to judge for themselves the validity of the Runcornian theory. For my part, if this procedure leads to the substantiation of Professor Runcorn's claims, I shall be the first to congratulate him, and would not only withdraw my criticisms

but in addition offer my sincere thanks for teaching me to regard nothing as impossible where modern geophysics is concerned.

So may we now all sit back, leaving the continents meanwhile to drift without their pilot, and look to Professor Runcorn to supply his computer program and relevant material, where-with to conclude this important matter.

R. A. LYTLETON

*St John's College,
Cambridge*

Received September 15, 1972.

¹ *Phil. Trans. Roy. Soc. A.*, **258**, 245 (1965).

² *The Observatory*, **91**, 164 (1971).

An Example of Hard-Water Error in Radiocarbon Dating of Vegetable Matter

THE hard-water effect is a recognized source of error in radiocarbon dating. It causes ages to be over-assessed and arises when the material to be dated, such as mollusc shell or plant, synthesizes its skeleton under water and so uses bicarbonate derived in part from old, inert sources. It is usually stated that the maximum possible error is equivalent to the half-life of ¹⁴C, 5,570 yr (Libby scale), but it rarely amounts to as much as this. Moreover, in an isolated dating it is not only impossible to evaluate, but even to know whether there is any over-estimation, but if the sample consists of tree wood, or the leaves, twigs or seeds of wholly terrestrial plants, then it can be assumed that no hard-water error is present.

I have recently studied, in company with Danish and British

with numerous thin black bands which contain moss fragments and sometimes small twigs. Band S1, from which came Birm-281 for radiocarbon dating (Figs. 1 and 2), when traced to the right, approached and ultimately coalesced with the Black Clay but before doing so changed its lithology to a dark sulphurous clay similar to the thick bed above it. From this point was collected Birm-288, the time equivalent of Birm-281; however, the dates ultimately appeared to differ. Amongst the numerous dark layers below S1 (Birm-281) were a group about 1.2 to 1.4 m lower which were contorted, possibly by cryoturbation but more probably by slumping down the slope of the basin. This is referred to later as the "contorted zone".

Clearing of the cliff about 20 m south of the main section revealed an irregular layer of woody peat about 60 cm below the Black Clay (Fig. 1, 282). Iversen¹ has described and investigated palynologically a peat in this position and the exposure seen in 1971 is almost certainly the same bed, eroded back some distance. In 1966, two radiocarbon dates (K962 and 963) were published on samples from this peat collected in 1949². This peat layer must pass towards the centre of the basin into one or more of the black bands in the lower sandy part of the succession and Krog from palynological considerations regards the Contorted Zone as its probable correlative.

The radiocarbon laboratory of the Geology Department of Birmingham University has dated eight samples from this section and the positions of these are shown in Fig. 1. There are in addition the two published dates from Copenhagen² which are assumed to come from about the same position as Birm-282.

In Fig. 2 these ten dates with their single standard deviation have been plotted against their position in the vertical succession. To allow for the condensation which occurs from left to right, dates such as 274 and 282 have been plotted in positions below the Black Clay where their respective horizons would be near the centre of the basin. It cannot be assumed that the

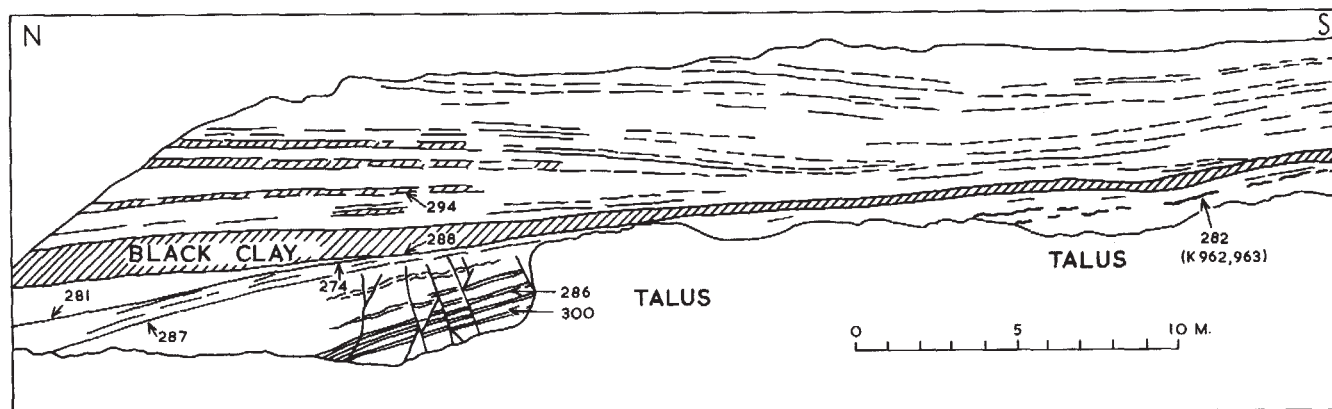


Fig. 1 Cliff Section at Nørre Lyngby, Jutland, Denmark.

colleagues, a fine section of late Quaternary sediments at Nørre Lyngby near the northern tip of Jutland. Fig. 1 shows a section of the cliff and reveals a sedimentary basin with its centre near to the left-hand (north) side of the figure with attenuation and condensation of the deposits to the right. Most prominent in the sequence is the band marked as Black Clay, a sapropelic sulphurous mud which contains many molluscs but very little in the way of macroscopic plant remains and no wood or twigs. From its contained pollen it has been equated with the Allerød zone¹ and so might be expected to yield a radiocarbon date older than 10,600 yr but certainly not exceeding 12,000 yr.

Above the Black Clay are alternate sands and grey silty clays, the latter devoid of large plant fragments and generally resembling the Black Clay except that they are less carbonaceous. Below this marker horizon the succession is largely sandy but

thicknesses in this succession are strictly proportional to time, but they are the best approach to this ideal.

First consideration of these dates shows a disturbing inconsistency, with several samples apparently much older than others below them, and with Birm-288 from a stratum confidently assigned to the Allerød giving a date which is certainly too high. Further consideration shows that, with the exception of Birm-287 which is intermediate, the dates group into two linear series.

Birm-281 was dated entirely on small twigs hand picked from layer S1. Birm-282 was a similar sample composed of pieces of wood washed from the peat. The Copenhagen dates K962 and K963 were also measured wholly or mainly on wood and with 282 form a confirmatory cluster, although there is no reason for believing that Birm-282 is the exact equivalent of either of the Copenhagen samples. Birm-300 consisted of a

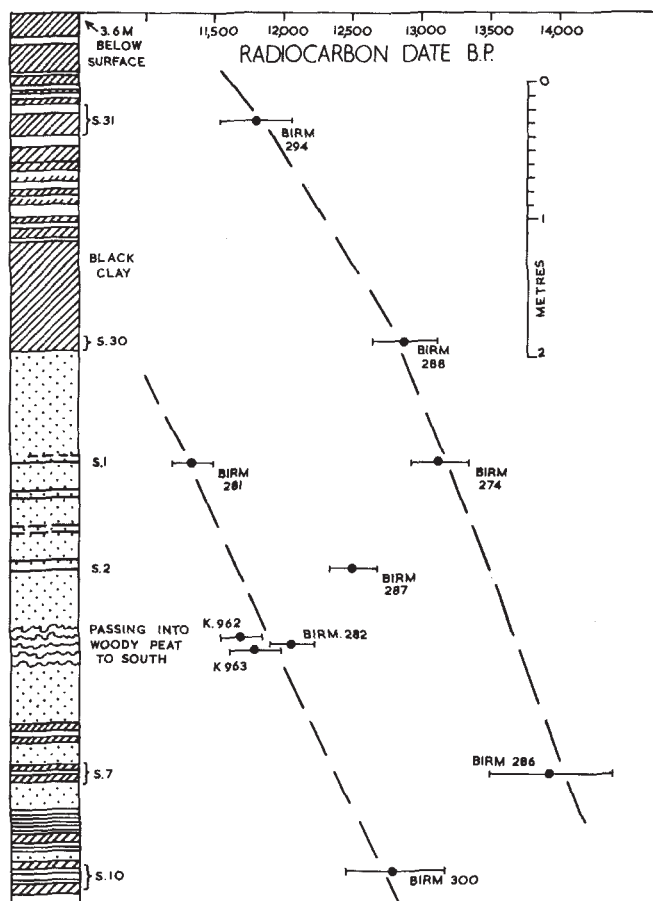


Fig. 2 Relation of ^{14}C dates to the succession at Nørre Lyngby.

mat of moss stems and leaves washed and sieved from the sand and, though small, appeared to be a satisfactory sample. The line drawn through the five dates mentioned above is likely, therefore, to be a valid time line.

In marked contrast to this group are Birm-294, 288, 274 and 286 which also form an almost linear series, approximately 1,700 yr older than the first. These "dates" do not fit the palynological evidence and are clearly over-estimates. Very striking is the difference between Birm-281 and 274 which are from the same band: the first consisted entirely of twigs; the second was a black mud. This and the two higher samples (288 and 294) are all of similar lithology. Before being gasified for counting, they were treated with acid to remove carbonate shells and sediment and then washed through a fine sieve which retained a fine-grained vegetable residue devoid of twigs, seeds or large leaves. It is probably largely algal and precisely the sort of material which, being sub-aqueous, would be expected to show hard-water error. Birm-286 was different in that the sieved residue included moss; the sample's apparent inclusion in the hard-water series could be explained if the moss were, for example, *Drepanocladus* which can develop successfully even though completely submerged.

It is not maintained that this demonstration of the hard-water effect is anything new. It is nevertheless of some interest to have a series of ^{14}C estimations from one succession which divide so obviously into two series, one reliable and one not, each related to the type of material being assayed, and where the size of the hard-water error can be roughly quantified.

F. W. SHOTTON

Department of Geology,
University of Birmingham, Birmingham B15 2TT

Received August 29, 1972.

¹ Iversen, J., *Medd. Dansk. Geol. Forening*, **10**, 130 (1942).

² Tauber, H., *Radiocarbon*, **8**, 213 (1966).

Occurrence of Nitride Nitrogen in Silicate Minerals

NITROGEN is one of the most abundant elements in the cosmos; in the Sun, only H, He, O and C exceed it¹. In the Earth's crust it ranks only as the thirty-first element in magmatic rocks², at a level of about 20 p.p.m. Because of the analytic difficulties in determining the nitrogen content of rocks little work has been done in this direction since Lord Rayleigh's determinations³, which gave nitrogen contents in the range of 28 to 68 p.p.m. for a variety of magmatic rocks. These values are confirmed by recent determinations⁴. Most workers assume, without further proof, that most or all nitrogen is present as the ammonium ion^{3,5}, substituting to a small extent for alkali ions in the minerals.

Certain classes of meteorites have a much higher nitrogen content⁶ than magmatic rocks from the Earth's crust: carbonaceous chondrites have 700 to 2,900 p.p.m. nitrogen, while enstatite chondrites contain 54–780 p.p.m. nitrogen, with a median value of 260 p.p.m., an order of magnitude higher than in rocks of the Earth's crust. The occurrence of organic materials is responsible for the high nitrogen abundance of carbonaceous chondrites. The relatively high nitrogen content in some enstatite chondrites is partly due to the presence of the mineral sinoite⁷, $\text{Si}_2\text{N}_2\text{O}$, which so far has been found only in two meteorites. Because, however, even enstatite chondrites, which do not contain sinoite or any other nitrogen mineral, can have nitrogen contents of up to 780 p.p.m., it is necessary to find a plausible mode of occurrence for the nitrogen.

Jack and Wilson⁸ have demonstrated recently that hot-pressing (at 1,700° C) of mixtures of $\alpha\text{-Si}_3\text{N}_4$ and Al_2O_3 yields solid solutions of alumina in silicon nitride. These are isostructural with $\beta\text{-Si}_6\text{N}_8$. This aluminum-silicon oxygen-nitride is given by them the formula $\text{Si}_{(6-0.75x)}\text{Al}_{0.67x}\text{N}_{(8-x)}\text{O}_x$, where x lies between 0 and 5. That such solid solutions can be prepared and the existence of natural⁷ and synthetic⁹ $\text{Si}_2\text{N}_2\text{O}$, and of solid solutions involving $\text{Si}_2\text{N}_2\text{O}^8$, means that the nature of the Si—N and Al—N bonds must be similar to the character of Si—O and Al—O bonds. In addition the sizes of N^{3-} and O^{2-} are similar¹⁰: $r_{\text{N}^{3-}}$ is 1.46 Å, if $r_{\text{O}^{2-}}$ is assumed to be 1.37 Å (the notation N^{3-} and O^{2-} applies to the formal oxidation numbers of these atoms, and does not assume ionic bonding). It is therefore possible that N^{3-} may substitute for O^{2-} in silicates, in a coupled substitution analogous to the one in silicon-aluminum oxygen-nitride⁸. It is also conceivable that nitrogen may be more abundant in the rocks of the Earth's mantle, because chemical reworking and degassing will not have occurred to the same degree as in the rocks of the Earth's crust. This proposal is in accord with the observations made on the nitrogen content of lunar basalts (Apollo 11) which show a nitrogen content approximately twice as high as Earth basalts¹¹.

Mason¹² has asked whether nitrogen in sinoite should be classified as siderophile or as lithophile. Based on the foregoing considerations it should be termed lithophile, associated with the silicates.

W. H. BAUR

Department of Geological Sciences,
University of Illinois at Chicago, Chicago, Illinois 60680

Received August 7; revised October 2, 1972.

¹ Goles, G. G., in *Handbook of Geochemistry* (edit. by Wedepohl, K. H.), **1**, chap. 5 (Springer, Berlin, 1969).

² Wedepohl, K. H., *Geochemie*, **75** (Gruyter, Berlin, 1967).

³ Lord Rayleigh, *Proc. Roy. Soc. Lond.*, **A**, **170**, 451 (1939).

⁴ Gibson, E. K., and Moore, C. B., *Anal. Chem.*, **42**, 461 (1970).

⁵ Stevenson, F. J., *Science*, **130**, 221 (1959).

⁶ Moore, C. B., in *Handbook of Elemental Abundances* (edit. by Mason, B.), **93** (Gordon and Breach, London, 1971).

⁷ Anderson, C. A., Keil, K., and Mason, B., *Science*, **146**, 256 (1964).

⁸ Jack, K. H., and Wilson, W. I., *Nature Physical Science*, **238**, 28 (1972).

- ⁹ Idrestedt, I., and Brosset, C., *Acta Chem. Scand.*, **18**, 1879 (1964).
¹⁰ Baur, W. H., in *Handbook of Geochemistry* (edit. by Wedepohl, K. H.), 2, chap. 7-A (Springer, Berlin) (in the press).
¹¹ Moore, C. B., Gibson, E. K., Larimer, J. W., Lewis, C. F., and Nichiporuk, W., in *Proc. Apollo 11 Lunar Science Conference* (edit. by Levinson, A. A.), 2, 1375 (Pergamon, New York, 1970).
¹² Mason, B., *Geochim. Cosmochim. Acta*, **30**, 365 (1966).

A Simple Microtensometer for Use in Scanning Electron Microscopes

STUDIES of service failures, such as fractures, are often inadequate because they are made after the event; in the laboratory failures can be observed as they happen. High speed photography allows study of crack propagation—Fig. 1 shows stages in cracking of tungsten at velocities of 2 km s^{-1} —but magnifications are limited. Less brittle solids fracture more slowly and allow high magnification studies; here I describe a technique which allows study of crack growth and other failure processes by scanning electron microscopy.

Many mechanical experiments have already been performed in scanning electron microscopes^{2,3}. Similarly, deformation stages have been used in transmission electron microscopy to observe the motion of dislocations in strain⁴ and the tearing of thin films⁵. In the present work the microtensometer was extremely simple (Fig. 2). The device uses a triangular wedge to produce a uniform strain; as the wedge (W) is driven forward the two sliding crossheads (C) are forced apart, thus applying the strain to the specimen (S) attached with screws to the sliding crossheads; the optimum specimen dimension is approximately $1.5 \text{ cm} \times 0.5 \text{ cm}$. Positioning and straining of the specimen are controlled from outside the chamber by universal joints and micrometer screws. The following three sequences show how the tensometers may be used.

Fig. 3 shows two micrographs in a sequence taken during the fracture of annealed copper foil (thickness 0.003 inch). The crack extended slowly and the foil was seen to thin ahead of the crack tip. No advance cracking occurred but tiny teeth formed in the mouth of the crack.

Fig. 4 illustrates the fracture of as-received molybdenum foil (thickness 0.001 inch). A notch was introduced by spark erosion and extended by fatigue. Fig. 4a shows the extension of the fatigue crack by the applied stress and b, c, d show the crack tip region. The laminar structure is quite apparent.

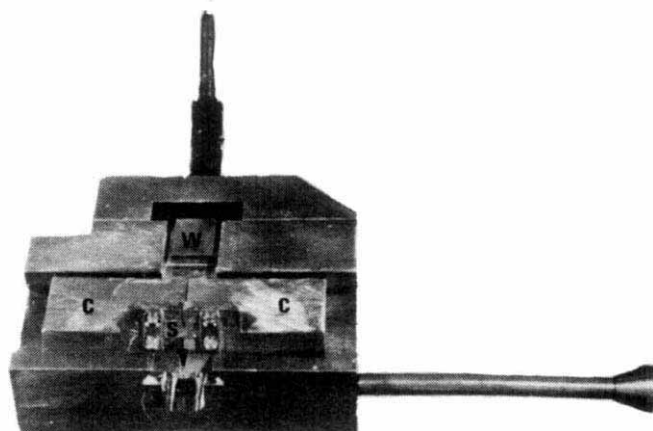


Fig. 2 Microtensometer for use in scanning electron microscope. S is the specimen, C the crosshead and W the wedge.

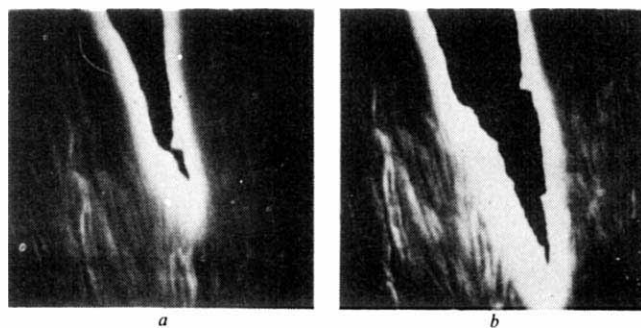


Fig. 3 Fracture of annealed copper foil using microtensometer. $\times 1,872$.

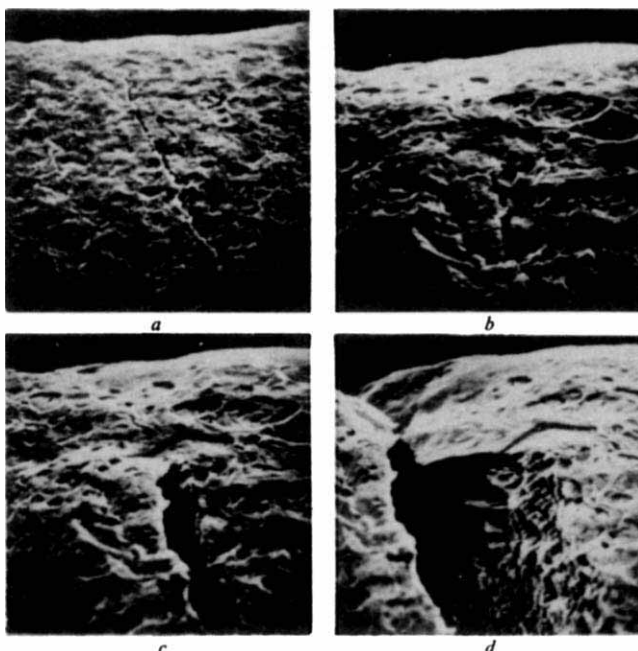


Fig. 4 Fracture of as-received molybdenum foil from fatigue crack. a, $\times 360$; b, c and d, $\times 1,440$.

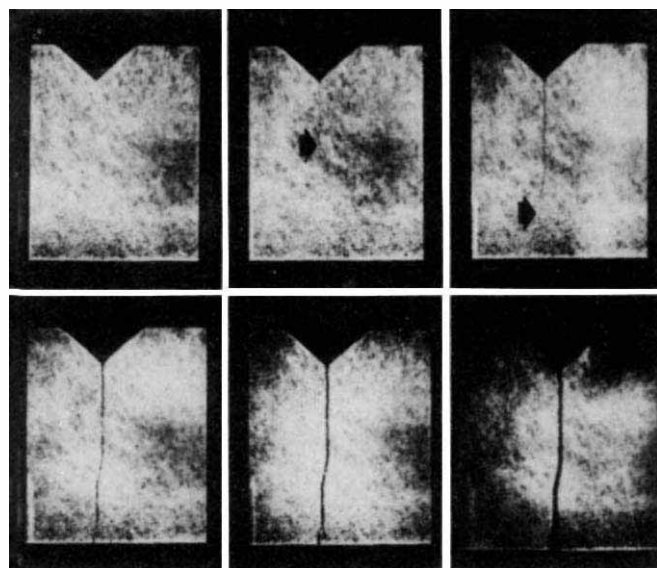


Fig. 1 The fracture of tungsten sheet filmed at $4 \mu\text{s}$ per frame. Crack speeds of 2 km s^{-1} have been recorded.

Fig. 5 illustrates the fracture of a recrystallized and etched molybdenum foil. The material was the same as that shown in the last sequence. Fig. 5a shows the spark-eroded notch before straining begins; b, c and d show thinning, formation of valleys and subsequent tearing. Although some intergranular cracks extending a short distance from the notch are visible these do not propagate; d, e and f show an intergranular crack

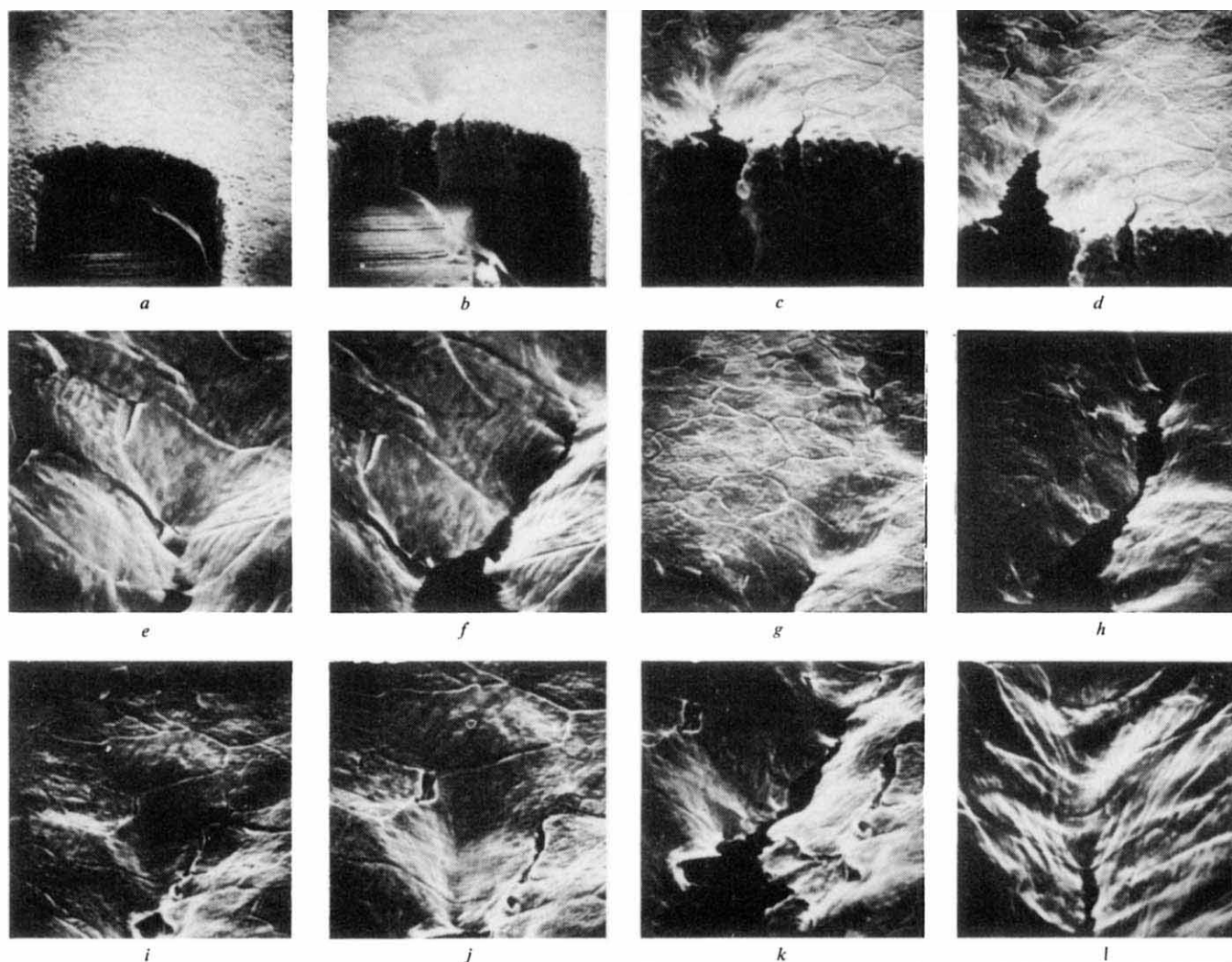


Fig. 5 Straining of recrystallized and etched molybdenum foil. *a*, $\times 207$; *b*, $\times 252$; *c* and *d*, $\times 387$; *e*, $\times 1,440$; *f*, $\times 1,170$; *g* and *h*, $\times 387$; *i*, $\times 450$; *j* and *k*, $\times 900$; *l*, $\times 1,170$.

well in advance of the main crack tip and this also does not propagate; *g*, *h* show the continuation of fracture by necking and tearing; *i*, *j*, *k* follow the passage of the main crack past a number of subsidiary advance cracks. Clearly the crack direction is determined by the reduction in cross-sectional area and not by the grain boundaries. Finally, *l* shows the extent of this reduction as well as the lines of plastic flow.

At present only slowly moving cracks can be followed in scanning electron microscopes owing to the limited time resolution. Because the electron gun is not bright enough to allow a faster scan, too few signal electrons per picture point are collected. Currently the minimum scan time is 40 m s^{-1} per frame and not until brighter electron sources are available will faster useful scanning be possible. Until such a time high speed fracture experiments cannot be considered. The time resolution of the transmission electron microscope is in theory superior to that of the scanner, being mainly limited by the time resolution of the recording equipment, but image intensification would be necessary to view rapidly changing events and this may impose a more stringent limitation. Therefore neither electron microscope can be used satisfactorily to follow fast fracture processes.

I have shown that a relatively simple device can be used to strain materials inside a scanning electron microscope and to allow study of deformation at high magnification. In the examples given it has been possible to follow the necking of material in the crack tip region and the growth of cracks in relation to the grain structure. The use of the microtensometer

is, of course, not confined to ductile metals and it has been used to study crack initiation in even the most brittle materials. Nor is it limited to polycrystals; single crystals of zinc were cleaved on the basal plane without difficulty.

I thank Dr J. E. Field for interest and encouragement and Mrs K. E. Singer for technical assistance.

H. S. DOBBS

Department of Applied Physical Sciences,
University of Reading

Received August 25, 1972.

¹ Dobbs, H. S., Field, J. E., and Heyes, A. D., *Proc. Eighth Int. Cong. High Speed Photography*, Stockholm, 435 (1968).

² Gane, N., and Bowden, F. P., *J. Appl. Phys.*, **39**, 1432 (1968).

³ Dingley, D. J., *Micron*, **1**, 206 (1969).

⁴ Forsyth, P. J. F., and Wilson, R. N., *J. Sci. Instrum.*, **37**, 37 (1960).

⁵ Pashley, D. W., in *Modern Developments in Electron Microscopy*, 226 (Academic Press, London and New York, 1964).

Appearance of Low-level Radioactive Silver on the World Silver Market

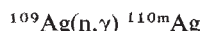
We have recently examined a number of bars of silver of East European origin for their radioactivity level. Out of a series of a hundred bars, we have radio-assayed seven bars picked at random. Engraved on the base of the bars are,

among other engravings, the weight in grams (on the average 11,100 g); the number 999 (ref. 8), most probably indicating the purity; and the number 1971, presumably the year of manufacture.

High resolution gamma-ray spectroscopy on the bars as a whole using Ge-Li detectors revealed lines of ^{108}mAg ($t_{1/2} = 127 \text{ yr}$)¹ and of ^{110}mAg ($t_{1/2} = 250.4 \text{ day}$)². No other activities could be detected. Because of the large extent of self-shielding in the bars themselves, absolute measurements have been performed on 20 g samples taken from different parts of one of the bars (Number 1), using a calibrated 3 inch square NaI(Tl) scintillation detector in a low-level counting set-up. The specific activities in these small samples are similar and the average is $0.084 \pm 0.010 \mu\text{Ci } ^{110}\text{mAg kg}^{-1}$ silver. This suggests a homogeneous distribution of the activity throughout the bar, a suggestion supported by the observation that the count rate of the bar as a whole is the same within 15% for all orientations.

To obtain a relative activity measure the seven bars mentioned have been compared as a whole, in identical geometry with respect to the Ge-Li detector. The results are given in Table 1. The ^{110}mAg activity per bar ranges from 0.9–1.8 μCi , and the ^{108}mAg activity is low, about 1.4% of the ^{110}mAg activity. Gamma ray activity, as counted with a Ge-Li detector placed in an anti-Compton array, showed that the activity ratio for the two isotopes is the same for two bars investigated. The value of this ratio as determined for a silver foil freshly irradiated with thermal neutrons, is 0.040% of ^{108}mAg relative to ^{110}mAg . On the basis of the difference between the ^{108}mAg percentage in the bars and the silver foil and the difference in half-life of the two isotopes, assuming that the silver from the bars has also been exposed to a flux of essentially thermal neutrons, we calculate that this must have happened an estimated $3.4 \pm 0.1 \text{ yr}$ before irradiation of the silver foil (September 8, 1972).

Our results indicate that the silver in the bars has been subjected to a neutron irradiation during late April 1969 $\pm$ 1 month. On the basis of a cross-section of 3.5 barn (ref. 3) for the slow neutron capture reaction



the dose is calculated to be $6.0 \times 10^{11} \text{ neutron cm}^{-2}$ for the most active bar and $3.4 \times 10^{11} \text{ neutron cm}^{-2}$ for most of the others investigated. Although as yet not all the bars under consideration have been inspected individually, a similar conclusion probably applies to all of them.

The explanation of this radioactivity may be one of the following possibilities.

First, the original ore containing the silver has been subjected to neutron irradiation after it was mined. This seems unlikely in view both of the amount of ore (several thousand tons of average grade ore already necessary to produce the hundred bars under discussion) and of the neutron dose of $10^{11} \text{ n cm}^{-2}$ involved.

Second, the silver bars themselves have been exposed to neutrons after manufacture. Apart from the lack of reasons for such irradiation, the apparent homogeneous activity distribution for all bars investigated does not support this possibility.

Third, the silver activity is introduced either intentionally or accidentally during the process of treating the ore and manufacturing the silver bars. This possibility cannot be ruled out altogether as only a total amount of about 1 mCi ^{110}mAg per 100 bars was involved at the date of manufacture (1971). It should be remembered that the silver activity used, under this assumption, was produced two years earlier (April 1969).

Fourth, the deposits from which the silver ore was derived have been mined by using an underground nuclear explosion. The date of April 1969 estimated for the time of formation of the radioactivity detected in the silver bars discussed is not

at variance with executed and planned nuclear detonations as described in the literature^{4,5}.

Table 1 ^{110}mAg Activity Registered in Seven Silver Bars

Bar No.	Counts* (658 keV γ)	μCi †
1	31,610	0.95
2	30,752	0.91
3	31,077	0.94
4	31,966	0.99
5	31,524	1.04
6	57,870	1.76
7	53,640	1.66

* Counts registered by a Ge-Li detector during a 1 h period underneath the 658 keV photopeak of ^{110}mAg .

† Approximate ^{110}mAg activity in μCi on September 1, 1972.

Although the level of radioactivity detected in the silver bars investigated is low (in fact a factor of 10 below the unrestricted safe-handling margin of $0.002 \mu\text{Ci g}^{-1}$ set in the Netherlands by law⁶) the information supplied above should be of general interest and of direct consequence, for instance, to those involved in low-level detection techniques (including photography) for different types of radiation.

This work is part of the research programme of the Institute for Nuclear Physics Research (IKO), made possible by financial support from the Foundation for Fundamental Research on Matter, and the Netherlands Organization for the Advancement of Pure Research. We thank Professor Dr A. H. Wapstra, director of the institute, for the interest shown for this investigation.

L. LINDNER
G. A. BRINKMAN
A. SCHIMMEL

*Instituut voor Kernfysisch Onderzoek,
Oosterringdijk 18, Amsterdam*

Received October 28, 1972.

¹ *Nuclear Data Sheets*, Section B, 7, No. 1, 55 (January 1972).

² *Nuclear Data Sheets*, Section B, 5, No. 5, 512 (May 1971).

³ BNL-325, *Neutron Cross-sections*, second ed., Suppl. No. 2 (1966).

⁴ Kedrovskiy, O. L., *UCRL-Trans-10477*, Lawrence Radiation Laboratory (1970).

⁵ Werth, G. L., *Nucl. Technol.*, **11**, 280 (1971).

⁶ Kernenergiewet, Uitvoeringsvoorschriften (C-2), art. 6, 1a (Staatsblad 404, September 10, 1969).

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.

The direct postal price per subscription is:

12 MONTHS* (52 issues per title)

	Surface mail UK and worldwide	U.S.A. and Canada
Nature (Friday)	£14	\$48
Nature + Nature Physical Science	£24	\$83
Nature + Nature New Biology	£24	\$83
All three editions	£29.50	\$108
Annual Index	£1	\$3

*Rates for shorter periods *pro rata* (minimum three months)
(Charge for delivery by air mail on application)

Deuterium-Hydrogen Ratio in Jupiter

Reeves and Bottinga, in discussing the D/H ratio in Jupiter¹, claim to make an estimate of this ratio based on theoretical work by Geiss and Reeves². In fact, the only value given is that which we have ourselves derived³ from a detailed reduction of the observational data of Beer *et al.*⁴. This result is incorrectly attributed to Black (to whom we had communicated our results) and is stated to be the D/H ratio in the CH₄ phase, which is also incorrect. Our result pertains to the overall isotopic ratio.

Furthermore, we are at a loss to understand how theoretical arguments based on tenuous considerations of chemical equilibria in the proto-solar nebula and carbonaceous chondrites were ever intended to lead to a value for the present-day D/H ratio in Jupiter.

R. BEER
F. W. TAYLOR

Space Sciences Division,
Jet Propulsion Laboratory,
California Institute of Technology,
Pasadena, California 91103

Received August 28, 1972.

- ¹ Reeves, H., and Bottinga, Y., *Nature*, **238**, 326 (1972).
- ² Geiss, J., and Reeves, H., *Astron. Astrophys.*, **18**, 127 (1972).
- ³ Beer, R., and Taylor, F. W., *Astrophys. J.* (in the press).
- ⁴ Beer, R., Farmer, C. B., Norton, R. H., Martonchik, J. V., and Barnes, T. G., *Science*, **172**, 1360 (1972).

Search for Plutonium-244 Tracks in Mountain Pass Bastnaesite

We have found that bastnaesite, a rare earth fluorocarbonate, from the Precambrian Mountain Pass deposit has an apparent Cretaceous fission track age, and hence does not reveal any anomalous fission tracks due to ²⁴⁴Pu.

Hoffman *et al.*¹ reported the first definite observation of ²⁴⁴Pu existing in nature when they isolated 2×10^7 atom of ²⁴⁴Pu from bastnaesite-rich ore from Mountain Pass, California. Because ²⁴⁴Pu has an 82 m.y. half life, its presence today, 56 half lives after the formation of the Earth, is a most impressive accomplishment which has raised questions as to whether the ²⁴⁴Pu could represent not primordial matter but later inflow of cosmic matter before the formation of the Mountain Pass deposit.

Because one of the methods of recognizing the decay of ²⁴⁴Pu is by observing the radiation damage tracks produced by its spontaneous fission^{2,3}, we have fission track dated^{4,5} the bastnaesite and accessory minerals from the adjacent rock at Schenectady and Denver, respectively, using methods described previously^{5,7}.

The bastnaesite was separated from the main carbonatite body, and the apatite and sphene were separated from two genetically related, contemporaneous⁸ shonkinite dikes. The bastnaesite was etched in 19% HCl at 55° C for 90 min using ~100 µm crystals. Calculated fission track lengths for CeFCO₃ are 16.8 µm; observed lengths extended up to 16.0 µm. This bastnaesite is characterized by an etching efficiency relative to Lexan polycarbonate of 0.62 (±0.07) and has a uranium concentration, as determined by induced fission tracks, of 21.2 (±1.8) p.p.m. by weight as compared to 18.1 (±1.0) p.p.m. by mass spectroscopy (F. Rourke, GE Knolls Atomic Power Laboratory, personal communication). Most of the tracks in the bastnaesite could be removed after one hour at 300° C (±20° C).

Table 1 shows that the age of the biotite (MP-21, 22) and sphene (MU-38) is approximately 1,400 m.y., but the fission track ages of the apatite and bastnaesite range from 50 to 80 m.y. These ages are consistent with the relative ease of thermally

Table 1 Ages of Mountain Pass, California, Samples

Material dated *	Dating method	Age (million years)	Rock type
Biotite (MP-21, MP-22)	K-Ar	1,440, 1,390 (ref. 6)	Shonkinite
Biotite (MP-21, MP-22)	Rb-Sr	1,380, 1,420 (ref. 6)	Shonkinite
Sphene (ML-16)	Fission track	1,170 ± 120 (2σ)	Shonkinite
Sphene (MU-38)	Fission track	1,400 ± 180 (2σ)	Shonkinite
Apatite (ML-16)	Fission track	53 ± 10 (2σ)	Shonkinite
Apatite (MU-38)	Fission track	60 ± 12 (2σ)	Shonkinite
Bastnaesite	Fission track †	78 ± 10 (2σ)	Carbonatite

* Sample localities:

MP-21, 22: Birthday Shaft.

ML-16: 7,250 ft. S50° W of Wheaton Springs, Calif.

MU-38: 11,250 ft. S80° W of Wheaton Springs, Calif.

Bastnaesite, Sulfide Queen carbonatite body.

† Total track counts from 18 different crystals.

removing tracks in apatite⁹ and bastnaesite, as compared with sphene and the presence of the 50 to 80 m.y. age "Laramide" orogenic event^{8,10}.

The nearly concordant dates for the bastnaesite and apatite indicate that there is no ²⁴⁴Pu fission track excess over the ²³⁸U track density. Given this low age, no such excess was to be expected. Therefore fission tracks cannot provide any support to the Hoffman *et al.*¹ reported observation of ²⁴⁴Pu in the Mountain Pass bastnaesite.

We thank F. Rourke for supplying the bastnaesite ore, R. T. Woods for experimental assistance, and B. S. Carpenter for neutron irradiations. The two samples of shonkinite were provided by J. C. Olsen.

R. L. FLEISCHER

General Electric Research and Development Center,
Schenectady, New York

C. W. NAESER

US Geological Survey,
Denver, Colorado

Received August 14, 1972.

- ¹ Hoffman, D. C., Lawrence, F. O., Merwherter, V. L., and Rourke, F. M., *Nature*, **234**, 132 (1971).
- ² Fleischer, R. L., Price, P. B., and Walker, R. M., *J. Geophys. Res.*, **70**, 2703 (1965).
- ³ Fleischer, R. L., Price, P. B., and Walker, R. M., *Geochim. Cosmochim. Acta*, **32**, 21 (1968).
- ⁴ Price, P. B., and Walker, R. M., *J. Geophys. Res.*, **68**, 4847 (1963).
- ⁵ Fleischer, R. L., and Hart, jun., H. R., *Proc. Burg Wartenstein Conf. Calibration of Hominoid Evolution* (Scottish Academic Press, Edinburgh, 1972).
- ⁶ Lanphere, M., *J. Geol.*, **72**, 381 (1964).
- ⁷ Naeser, C. W., and Dodge, F. C. W., *Bull. Geol. Soc. Amer.*, **80**, 2201 (1969).
- ⁸ Olsen, J. C., Shawe, D. R., Pray, L. C., and Sharp, W. N., *US Geol. Survey Prof. Paper*, **261** (1954).
- ⁹ Naeser, C. W., and Faul, H., *J. Geophys. Res.*, **74**, 705 (1969).
- ¹⁰ Naeser, C. W., *J. Geophys. Res.*, **76**, 4978 (1971).

Ventilation Inception on Surface Piercing Foils or Struts

VENTILATION is a problem of long standing associated with high speed hydrofoil craft. The sub-atmospheric pressures, generated by the movement of the foils through the water, can cause air to be drawn from above the free surface to form a stable or semi-stable cavity attached to the foil. When associated with lifting foils, ventilation causes a marked loss of lift and in other instances can result in loss of directional stability.

The existence of a region of separated flow has been cited as a requirement before ventilation can take place on a foil¹ and recent studies have confirmed this view (B. N. Cole and our work, in preparation). Nevertheless it has also been shown²



Fig. 1 Appearance of underside of free water surface showing growth of perturbations just before ventilation inception. Flow was from left to right at 10 foot s^{-1} and the foil was an NACA 16-021 at 20° incidence angle.

that for a surface piercing foil air does not immediately or invariably enter a low pressure separated region, apparently because of a thin layer of non-separated fluid in the vicinity of the water surface which effectively resists rupture.

Experiments in a high speed recirculating water channel, using stroboscope illumination and high speed cine techniques, have shown how the surface "seal" breaks down to admit air to the separated regions and initiate ventilation. As the water flows over the strut the free surface is drawn down on the low pressure face by the pressure gradient. This downward acceleration causes any small disturbance of the water surface ahead of the strut to grow in a manner analogous to the growth of perturbations in thin sheets of water as described by Taylor². Once past the foil, the perturbations collapse rapidly, leaving vortices whose paths can often be followed by the presence of air filled cores. As the water speed or angle of incidence of the foil is increased, the perturbations eventually reach a size at which the surface layer is ruptured, air is admitted to the separated regions and the cavity forms. The appearance of the surface just before rupture can be seen in Fig. 1. Downward accelerations of the surface of about $40g$ have been noted just before ventilation and the time for the cavity to form once the surface has been breached is of the order of 0.4 s .

Because the ultimate size of the perturbations depends on their initial amplitude, the ventilation inception point is largely determined by the nature of the free surface presented to the foil. This observation no doubt explains some of the anomalies

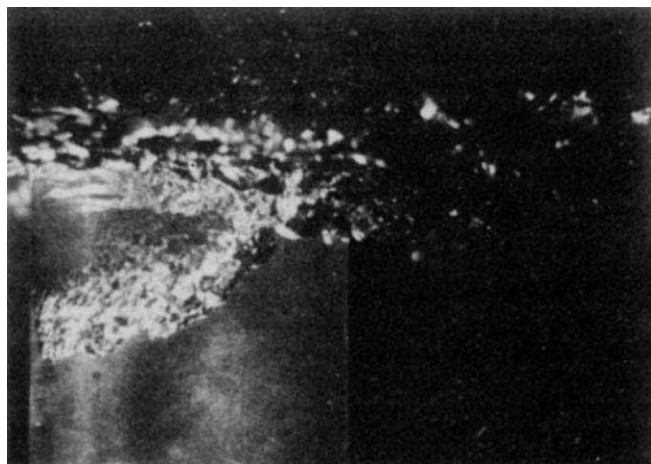


Fig. 2 With this sharp nosed biogival foil at 9° incidence, the surface has been breached at the nose, and air is travelling down and across the foil. Flow is again from left to right, at 5 foot s^{-1} .

previously noted when towing tank tests have been compared to the results of sea trials.

Occasionally another mode of rupture was observed, in which air entry occurred at the nose (Fig. 2). This was found only in thin foils which exhibited a separated nose bubble. Air entry at this point appears to be associated with a vortex of considerable strength inside the bubble and large amplitude surface perturbations, and was less predictable than the mode previously described. Once started, cavities would form rapidly, usually in less than 0.1 s .

We thank the Defence Research Establishment, Atlantic, Halifax, Nova Scotia, for funds.

A. WRIGHT
P. D. SWALES
R. C. MCGREGOR

*Mechanical Engineering Department,
University of Leeds*

Received July 5, 1972.

¹ Wadlin, K. F., *Mechanics of Ventilation Inception*, 425, Second ONR Symp. Naval Hydrodynamics (1958).

² Taylor, G. I., *Proc. Roy. Soc.*, **253**, A, 289 (1959).

BIOLOGICAL SCIENCES

Infection of Protoplasts from Yeast with Tobacco Mosaic Virus

PROTOPLASTS from higher plant cells can become infected with plant viruses under special conditions¹⁻³, and it has been shown that yeast cells can become infected with viruses normally found in different fungal genera⁴. We thought that naked yeast cells (yeast protoplasts) might provide a suitable system for demonstrating the infection of yeast cells with a virus such as tobacco mosaic virus (TMV). We report here how we infected yeast protoplasts with TMV and measured its multiplication in them.

A common strain of *Saccharomyces cerevisiae* was grown for 24 h at 25°C in 3 l. flasks containing 250 ml. of a liquid media⁵, on a rotary shaker. Approximately 1 g "wet weight" of yeast cells were collected by centrifuging ($1,500g$), washing twice by resuspending in distilled water, and recentrifuging. The yeast cells were then suspended in 20 ml. of 0.28 M mercaptoethylamine HCl, osmotically stabilized with 0.7 M sorbitol and 0.01 M citric acid, pH 6.5. The suspension was incubated for 20 min at 30°C with occasional shaking. Yeast cells were collected by centrifugation ($1,000g$), and resuspended in 2 ml. of snail-gut enzyme (Helicase) at a concentration of 30 mg ml^{-1} . The suspension was incubated for 1 h at 30°C , with occasional shaking, when phase contrast microscopy revealed yeast protoplasts. The enzyme and cell wall debris were removed by centrifuging ($1,000g$) in a 'Ficoll' density gradient, and the protoplasts were finally suspended in fresh osmotic stabilizer (sorbitol-citric acid).

Samples of the protoplast suspension were mixed with equal volumes of a virus suspension containing $250 \mu\text{g ml}^{-1}$ of purified TMV (Rothamsted common strain), previously diluted in the osmotic stabilizer and preincubated for 10 min at 28°C with reciprocal shaking. The protoplast/virus mixture was kept at 28°C for 90 min with continuous reciprocal shaking.

The protoplasts were centrifuged and divided into aliquots containing $1-5 \times 10^7$ protoplasts. Each aliquot was centrifuged and resuspended twice in the osmotically stabilized incubation medium⁵ to remove any unadsorbed virus. The zero time aliquot was stored at 4°C . The remaining aliquots of inoculated protoplasts were diluted in 10 ml. of the incubation medium to give approximately $1-5 \times 10^6$ yeast protoplasts ml^{-1} and incubated at 25°C in diffuse light on a rotary shaker. After

set times of incubation, aliquots of infected protoplasts were removed and stored at 4° C. The protoplasts were extracted² and virus infectivity assayed by lesion counts in *Nicotiana tabacum* cv Xanthi n.c., using carborundum as an abrasive. The infectivity in the extracts of the different samples was compared with the zero time sample. The results of several experiments show that after 2–3 h incubation the amount of the infectivity of the protoplasts had decreased below the amounts found in the zero time samples. Later samples produced more lesions, most after 17–24 h incubation. The infectivity declined to approximately 50% of the maximum in 36, 48 and 72 h samples. To give the results of one experiment, the total number of lesions from 7 half leaves were 42, 19, 570, 386, 379 and 218 respectively for the 0, 2½, 17, 24, 48 and 72 h samples. This is when the extracted fluid was inoculated undiluted. When the extracted fluid was inoculated at dilution 1/10 the number of lesions were 20, 9, 117, 72, 113 and 74 respectively for the same samples. Infectivity assays of ten-fold dilutions of the zero time extracts indicated that an inhibitor was present, because they produced as many or more lesions than undiluted extracts. We are trying to determine the mode of inhibition and how to overcome it. During the 24 h incubation the infectivity of the protoplast extract increased 15–40 times compared with lesion counts from zero time samples.

Fungi can act as vectors of certain plant viruses⁶ and recently TMV strains have been extracted from conidia of powdery mildews from oak and barley⁷.

The present results show the versatility of TMV. Although the infectivity of the inoculated protoplasts could increase by 40-fold, the actual amount of virus produced by the protoplasts is very small. The amount of fungal double-stranded RNA virus found in infected yeast cells⁴ suggests that our technique can be improved and we are trying to do so.

We thank Dr A. Weimken for advice on the isolation of yeast protoplasts. R. H. A. C. receives a Science Research Council cooperative award in pure science.

R. H. A. COUTTS
E. C. COCKING

Department of Botany,
University of Nottingham,
University Park,
Nottingham NG7 2RD

B. KASSANIS

Rothamsted Experimental Station,
Harpenden,
Hertfordshire

Received August 26, 1972.

- ¹ Takebe, I., and Otsuki, T., *Proc. US Nat. Acad. Sci.*, **64**, 843 (1969).
- ² Coutts, R. H. A., Cocking, E. C., and Kassanis, B., *J. Gen. Virol.* (in the press).
- ³ Coutts, R. H. A., in *Viruses in Isolated Protoplasts—a Potential Model System for Studying Nuclear Protein Replication* (Colloques Internationaux Versailles, 1972).
- ⁴ Lhoas, P., *Nature New Biology*, **236**, 86 (1972).
- ⁵ Darling, S., Theilade, J., and Birch-Andersen, A., *J. Bact.*, **98**, 797 (1969).
- ⁶ Grogan, R. G., and Campbell, R. N., *Ann. Rev. Phytopathol.*, **4**, 29 (1967).
- ⁷ Nienhaus, F., and Yarwood, C. E., *Phytopathology*, **62**, 313 (1972).

Conjecture on the Spread of Infection in Two Dimensions Disproved

Williams and Bjerknes^{1,2} conjectured, on the basis of computer simulations, that in the spread of abnormal cells through a plane lattice, the boundary of the affected area is of dimension greater than one. If true, this would be of considerable interest as an example of a population growth model whose behaviour in two dimensions is radically worse than in one, because it would imply, as I show here, that the affected area

expands with asymptotically infinite velocity. Comparison with a simple epidemic model to which one can apply a result of Hammersley's percolation theory³, however, shows that the velocity of expansion is bounded, and hence that the conjecture must be false.

Problems of spatial spread of or through a population are naturally two-dimensional for the most part. Thus, though there exist environments such as seashores and railway embankments which are essentially one-dimensional, the principal justification for work on one-dimensional models of, for instance, the spread of an advantageous gene⁴ or of epidemics^{5–7} is as a step towards the analysis of the more difficult two-dimensional cases. Brief studies^{7,8} suggest that results on deterministic models can be extended from one dimension to two, but there is little evidence on the more interesting question whether similar extensions are possible for stochastic models. In this context the conjecture of Williams and Bjerknes^{1,2}, that in the spread of abnormal (tumour) cells through a plane lattice, the boundary of the affected area is of dimension greater than one, deserves attention. If this were true, it would be possible to deduce that the velocity of spread of the affected area is asymptotically infinite; and one might expect to be able to extend this surprising result to two-dimensional stochastic models for the spread of other phenomena, such as those mentioned above.

In the model considered by Williams and Bjerknes², the basal layer of the epithelium is regarded as a regular lattice of hexagonal cells. If these cells are of unit side the length ($\partial(t)$) of the boundary between the abnormal and normal cells at time t is equal to the number of adjacent (normal, abnormal) pairs. For each such pair there is probability αdt that the normal will be replaced by a daughter of the abnormal in a time-interval of length dt , and βdt that the reverse will occur ($\alpha/\beta = \kappa > 1$, the carcinogenic advantage). The case where $\beta = 0$ ($\kappa = \infty$) is identical to the simple epidemic model with only nearest-neighbour infection; as we shall see below, percolation theory can be applied to this simpler model.

Let $N(t)$ be the number of abnormal cells ($= 1$ at time 0), and $r(t)$ the radius of a circle of area equal to that of the abnormal cells, that is $(\alpha N(t)/\pi)^{1/2}$, where α is the area of a regular hexagon of unit side. Then Williams and Bjerknes's conjecture is that, in those cases when the tumour does not regress,

$$\partial(t) \sim r(t)^{1+\epsilon}, \quad \epsilon(\approx 0.1) > 0 \quad (1)$$

If we define the crinkliness, $c(t)$, to be the ratio $\partial/2\pi r$ of the boundary length to the minimum possible for a like area, the conjecture becomes

$$c(t) \sim r(t)^{\epsilon}, \quad \epsilon(\approx 0.1) > 0 \quad (2)$$

A short non-rigorous argument starts with

$$\frac{dN}{dt} = (\alpha - \beta)\partial \quad (3)$$

(compare Williams and Bjerknes's equation (1)², which should read $dN/dt = (\kappa - 1)\partial$, not " $= (\kappa - 1)\pi$ "), and deduces " $dr/dt \sim c$ "; from which, if $c(t) \rightarrow \infty$ as $t \rightarrow \infty$, so does r/t . One can properly deduce, however, only that

$$\begin{aligned} \frac{1}{dt} E(r(t+dt) - r(t) | \partial(t)) &\approx \\ \frac{\alpha}{2\pi r t} E(N(t+dt) - N(t) | \partial(t)) &= (\alpha - \beta)a c(t) \end{aligned} \quad (4)$$

To obtain a rigorous connexion between the asymptotic values of r/t and c we must investigate the process of tumour growth in more detail, isolating the difficulties inherent in the dependent sequence of values of the random variable $\partial(t)$.

The growth of a tumour occurs as a sequence of happenings each of which consists of the replacement of a normal by an abnormal cell or *vice versa*. These happenings form a simple

random walk on the integers, with probability $\alpha/(\alpha+\beta)$ of an increase, $\beta/(\alpha+\beta)$ of a decrease, at each step; and with the state 0 as an absorbing barrier. It is thus a standard result that, in the cases when the tumour does not regress, $N(m)/m \rightarrow (\alpha-\beta)/(\alpha+\beta)$ with probability 1 as m tends to infinity. Note that the size and shape of the affected area have not yet entered the discussion; they only matter when we consider the expected time-interval between successive happenings. Since the number of adjacent (normal, abnormal) pairs is equal to the boundary length ∂ , the distribution of the time to the next happening after time t is the convolution of $\partial(t)$ negative exponential distributions of parameter α , and $\partial(t)$ negative exponential distributions of parameter β . It is thus easy to show (using the strong law of large numbers) that the ratio of the time of the m th happening, $t(m)$, to its expected value $\sum_{i=1}^m [\alpha+\beta]\partial(i)^{-1}$ tends to 1 with probability 1, for any particular (fixed) sequence of values of ∂ .

Let $\Phi(m) \equiv (\alpha+\beta)N(m)/m(\alpha-\beta) \rightarrow 1$ as $m \rightarrow \infty$. Then the average velocity of expansion of the tumour to the m th happening

$$\frac{r(m)}{t(m)} = \frac{2(\alpha-\beta)a\Phi(m)^{\frac{1}{2}}m^{\frac{1}{2}}}{\sum_{i=1}^m \Phi(i)^{-\frac{1}{2}}i^{-\frac{1}{2}}c(i)^{-1}} \quad (5)$$

The crinkliness has bounds $1 \leq c(i) \leq O(i^{\frac{1}{2}})$, so we can compare the denominator with " $x^{-\frac{1}{2}}c(x)$ " to obtain

$$\text{if } c \rightarrow c^* \text{ (as } m \rightarrow \infty), \frac{r}{t} \rightarrow (\alpha-\beta)ac^* \quad (6)$$

In general, it is difficult to analyse the dependent sequence of random variables $(c(m))$ (or equivalently $(\partial(m))$); however, Williams and Bjerknes's conjecture deals with precisely this sequence, implying (equation (2)) that $c(t) \rightarrow \infty$ as $t \rightarrow \infty$, and hence (since $m \rightarrow \infty$ as $y \rightarrow \infty$) that

$$\frac{r}{t} \rightarrow \infty \text{ as } t \rightarrow \infty \text{ with probability 1} \quad (7)$$

Thus their conjecture will be disproved if it is shown that r/t is bounded.

It seems intuitively clear that the growth of the affected area will be fastest, for given α , in the simple case where $\beta=0$. This notion can be made rigorous using (for instance) my theory (section 3.2 of ref. 7) following which we would define a single probability space both for the tumour model with parameters α and β and for the simple epidemic with parameter α . This probability space consists of the product of poisson processes of frequencies α and β for each (ordered) adjacent pair of cells in the lattice. Then, given the obvious "rules of infection", the set of abnormal/infected cells is well defined for almost all points ω of the sample space. Because the rules of infection are strictly more demanding for the tumour model, the set of abnormal cells $S_b(\omega, t)$ is a subset of the set of infected $S_0(\omega, t)$ for all such ω (and for all t); and hence any upper bound on r/t for the simple epidemic is also an upper bound, with probability 1, for the tumour model of equal α (for all β).

Now the simple epidemic with only nearest-neighbour infection is a special case of the percolation models considered by Hammersley and Welsh^{3,9}. Hammersley³ defines $M(\omega, t)$ as the number of steps necessary to reach the furthest infected cell at time t ; if $r^*(\omega, t)$ denotes the distance to this cell, $\frac{1}{2}M \leq r^* \leq M\sqrt{3}$, so that $r(\omega, t) \leq M(\omega, t)\sqrt{3}$. He proves that M/t tends to a finite limit C as $t \rightarrow \infty$, and that $C \leq \alpha\xi$, where $\xi \exp 1/\xi = e^{1+k}$ and k is the connective constant³ of the lattice, ≈ 1.5 in this case. Thus for all α and β

$$\limsup_{t \rightarrow \infty} \left(\frac{r}{t} \right) \leq C\sqrt{3} \leq \alpha\xi\sqrt{3} \approx 18\alpha \quad (8)$$

which contradicts relation (6), and therefore completes our proof that the dimension of the boundary cannot be greater than 1 (and, since it cannot be less, it must equal 1).

Lastly we note that relation (7) implies that the crinkliness has an upper bound, which tends to ∞ as $\kappa \downarrow 1$; comparison with (5) yields

$$\limsup_{t \rightarrow \infty} c(t) \leq \frac{\alpha C \sqrt{3}}{(\alpha-\beta)a} = \frac{2}{3} \left(\frac{\kappa}{\kappa-1} \right) C < 7 \left(\frac{\kappa}{\kappa-1} \right) \quad (9)$$

It has been shown above that the velocity of expansion of the affected area depends linearly on the crinkliness c , and that c is bounded. It remains possible that $c(t) \propto N^{0.05}$, as Williams and Bjerknes conjecture, for small enough values of N ; though the work of Morgan and Welsh¹⁰ on a similar model suggests that c is likely to tend fairly quickly to a limit c^* , and to be concave towards the origin, as one might well expect.

If indeed c tends fairly quickly to c^* , and if as one might expect, and as Williams and Bjerknes's simulations (Fig. 1 of ref. 2) confirm, c^* depends inversely on κ , then the crinkliness of a tumour which is known to have a single origin would provide a direct and reliable means of estimating the carcinogenic advantage (κ).

One further surprising remark of Williams and Bjerknes deserves comment, namely that the mean first-passage time to a total of N abnormal cells is approximately $1.25N^{0.45}/(\kappa-1)$ independent of the value of κ (equation (2), ref. 2). This would imply that the crinkliness $c \approx 1.87N^{0.05}$ independent of κ , whereas their simulations (Fig. 1 of ref. 2) show, as mentioned above, higher values of c when κ is near 1; for example, when $N=400$, $c \approx 2.54$ for $\kappa = \infty$, ≈ 6.06 for $\kappa = 1.1$. It is hard to see how this can be reconciled with their equation (2).

I should like to draw attention to the theoretical questions which remain unanswered. The bound we have found for $\limsup c(t)$ (equation (9)) tends to ∞ as $\kappa \downarrow 1$; does $\limsup$

$c(t)$ itself tend to ∞ as $\kappa \downarrow 1$? A question of rather wider implications is whether there exist two-dimensional infection models for which the dimension of the boundary can be greater than one. Certainly this would require that the crinkliness and the velocity of expansion should be asymptotically infinite; and there must be a possibility of "infection at a distance", indeed at arbitrarily large distances, since otherwise one can use percolation theory as here to obtain bounds for the velocity of expansion.

DENIS MOLLISON

King's College Research Centre,
Cambridge

Received June 22; revised August 9, 1972.

- ¹ Williams, T., and Bjerknes, R., *Adv. in Appl. Prob.*, **3**, 210 (1971).
- ² Williams, T., and Bjerknes, R., *Nature*, **236**, 19 (1972).
- ³ Hammersley, J. M., *J. Roy. Statist. Soc.*, **B**, **28**, 491 (1966).
- ⁴ Kolmogoroff, A. N., Petrovsky, I. G., and Piscounoff, N. S., *Bull. Univ. Etat Moscou (Sér. Intern.)*, **A**, **1**, 6, 1 (1937).
- ⁵ Kendall, D. G., *Mathematics and Computer Science in Biology and Medicine*, 213 (HMSO, London, 1965).
- ⁶ Mollison, D., *Adv. in Appl. Prob.*, **4**, 233 (1972).
- ⁷ Mollison, D., *Proc. Sixth Berkeley Symp. on Math. Statist. and Prob.*, **3**, 579 (University of California Press, in the press).
- ⁸ Kendall, D. G., *J. Roy. Statist. Soc.*, **A**, **120**, 64 (1957).
- ⁹ Hammersley, J. M., and Welsh, D. J. A., *Bernoulli-Bayes-Laplace Anniversary Volume*, 61 (Springer-Verlag, Berlin, 1965).
- ¹⁰ Morgan, R. W., and Welsh, D. J. A., *J. Roy. Statist. Soc.*, **B**, **27**, 497 (1965).

Lamellar Transformation of Lung Mitochondria under Conditions of Stress

THE lung surfactant is rich in dipalmitoyl lecithin¹ which lowers the surface tension of the lung lining to prevent collapse and transudation^{2,3}. Strong evidence suggests it originates in the lamellated osmophilic bodies (LOPBs) of the Type II

cells of the alveoli^{4,5}, which are often of spiral form. Some workers^{4,6,7} have regarded the LOPBs as transformed mitochondria; but others do not agree⁸⁻¹¹. Under conditions of stress many of the mitochondria of the Type II cells transform into spiral bodies with the 4 nm layering, affinity for lead ferricyanide^{5,12} and sensitivity to damage by alcohol and epoxypropane⁵ which are characteristic of the LOPBs. When the transitional bodies are found in a specimen, they are present (as in the tissue for Figs. 1 and 2) in almost every Type II cell. They occur in about 1 in 20 untreated animals.

Pieces of lung, fixed in 2% glutaraldehyde and 1% osmium tetroxide, were further fixed and stained in a mixture of 10 vol M/40 lead nitrate and 10.5 vol M/60 potassium ferricyanide solutions. They were embedded for electron microscopy in 'Araldite' with acetone as dehydrator and thinner and a quick time-schedule⁵. The transformation was originally found in rats which had received a very high dosage ($Ct=120,000$ mg min m^{-3} over 1 h) of *o*-chlorobenzylidene malononitrile (CS) smoke. Subsequent work showed that the transformation was not a specific effect of the chemical, as the same effects were found with a rat which had been kept at 3°-4° C for 16 h. This suggested an adrenal cortical influence on the transformation, and a test was made with 9-fluoro prednisolone acetate (9FP). Rats treated with this showed numerous transitional bodies. Fig. 1 shows such a body from a rat 3 days after intramuscular injection of 2.5 mg kg^{-1} of 9FP. At high magnification the dense portion of the body showed 4 nm spacing; the positions at which this was visible shifted as the specimen was tilted in the electron beam, thus demonstrating a layered structure. A few mitochondrial cristae survive in the body.

Part of the same cell with a normal mitochondrion, part of a normal LOPB, and four bodies in various stages of transition are shown in Fig. 2. The sequence of transformation first involves a lightening of the background of the mitochondrion, with some swelling and disappearance of some cristae. One or more of the cristae then appear to roll into scrolls, which become denser, and coalescence of the walls of the cristae and of adjacent turns of the scroll gives rise to a 4 nm spacing. The paler portions represent the inner parts of the membrane of the crista, which in turn originates from the inner bounding membrane of the mitochondrion⁷. A monolayer of surfactant thus has its origin as one half of such a membrane. The remaining cristae then disappear, and the scrolls grow until a normal LOPB results. These findings do not exclude other origins for some LOPBs such as the multivesicular bodies¹⁰.

The proliferation of mitochondrial and other membranes to form dense scrolls^{13,14}, in one case induced by a cyanoketo-



Fig. 1 Mitochondrion forming scroll in Type II cell of rat lung after injection of 9FP. Scale bar 250 nm.

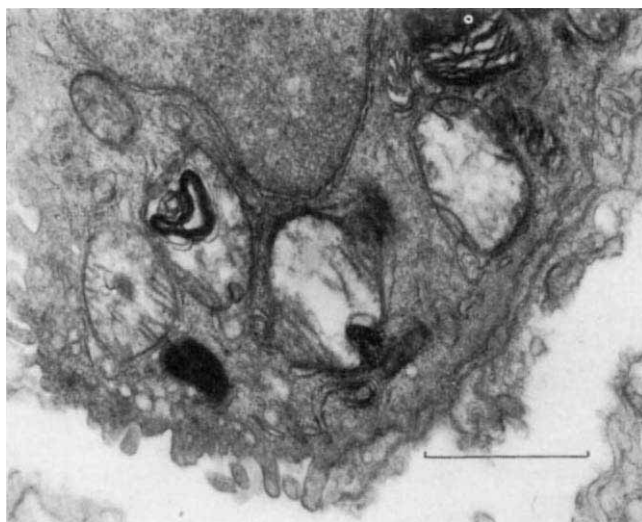


Fig. 2 View of same cell showing normal mitochondrion, normal LOPB, and four intermediate bodies. Scale bar 1 μ m.

steroid¹⁵, has been reported from many non-pulmonary sites. The figures shown suggest that these bodies are less affected by embedding fluids than are the pulmonary bodies; this may be due to a higher content of unsaturated phospholipid, and better fixation by osmium tetroxide¹⁶. Sacktor and Shimada¹⁴ report their presence in the flight muscle of the ageing blowfly and regard it as a degenerative change. We have found 4 nm spacing in bodies similar to those reported by Candiolo and Filogamo¹³ in the neural tube of the chick embryo.

There is evidence¹⁷ that in the early stages of the respiratory distress syndrome of the newborn (RDS), in which surfactant is deficient, LOPBs are also deficient. Kotas and Avery¹⁸ have accelerated the production of surfactant in foetal rabbits by treatment with 9FP. Gandy *et al.*¹⁹ report a case of RDS in a full-term baby with adrenal hypoplasia. The present findings suggest that 9FP acts to stimulate the transformation of mitochondria into bodies of surfactant; whether RDS involves a delay of surfactant production at this particular stage is not yet known.

R. E. PATTLE
C. SCHOCK
P. DIRNHUBER
J. M. CREASEY

Chemical Defence Establishment,
Porton Down,
nr. Salisbury, Wiltshire

Received April 12; revised September 20, 1972.

- ¹ Brown, E. S., *Amer. J. Physiol.*, **207**, 402 (1964).
- ² Pattle, R. E., *Nature*, **175**, 1125 (1955).
- ³ Pattle, R. E., *Physiol. Rev.*, **45**, 48 (1965).
- ⁴ Kiaus, M., Reiss, O. K., Tooley, W. H., Piel, C., and Clements, J. A., *Science*, **137**, 750 (1962).
- ⁵ Pattle, R. E., Schock, C., and Creasey, J. M., *Experientia*, **28**, 286 (1972).
- ⁶ Schulz, H., *Virchow's Arch.*, **328**, 582 (1956).
- ⁷ Woodside, G. L., and Dalton, A. J., *J. Ultrastruct. Res.*, **2**, 28 (1958).
- ⁸ Karrer, H. E., *J. Biophys. Biochem. Cytol.*, **2**, 241 (1956).
- ⁹ Campiche, M. A., Gautier, A., Hernandez, E. I., and Reymond, A., *Pediatrics*, **32**, 976 (1963).
- ¹⁰ Sorokin, S. P., *J. Histochem. Cytochem.*, **14**, 884 (1967).
- ¹¹ Niden, A. H., *Science*, **158**, 1323 (1967).
- ¹² Dermer, G. B., *J. Ultrastruct. Res.*, **33**, 306 (1970).
- ¹³ Candiolo, L., and Filogamo, G., *Z. f. Zellforsch.*, **69**, 480 (1966).
- ¹⁴ Sacktor, B., and Shimada, Y., *J. Cell. Biol.*, **52**, 465 (1972).
- ¹⁵ Finegold, M. J., and Greene, L. E., *J. Cell. Biol.*, **45**, 455 (1970).
- ¹⁶ Cope, G. H., and Williams, M. A., *J. Microsc.*, **90**, 31 (1969).
- ¹⁷ Gandy, G., Jacobson, W., and Gairdner, D., *Archs. Dis. Childh.*, **45**, 289 (1970).
- ¹⁸ Kotas, R. V., and Avery, M. E., *J. Appl. Physiol.*, **30**, 358 (1971).
- ¹⁹ Gandy, G., Bradbrooke, J. G., Naidoo, B. T., and Gairdner, D., *Archs. Dis. Childh.*, **43**, 8 (1968).

Cadmium-induced Testicular Injury and Alterations of Androgen Synthesis in Brook Trout

NON-LETHAL doses of cadmium salts can cause specific injury to the testis of various mammalian species^{1,2}. There is some evidence that the spermatogenic process can be adversely affected by Cd, possibly through its injurious effects on the vasculature of the testis^{3,4}, but little is known of the effects of Cd on androgen synthesis⁵. In view of the well-documented reports of a direct relationship between androgens and maintenance of spermatogenesis in several vertebrate species including fish⁶ we investigated the effects of Cd on the testis of a freshwater-maintained salmonid fish, the brook trout *Salvelinus fontinalis*.

We observed definite testicular injury and changes in androgen synthesis in two groups of 3–4 year old brook trout treated with CdCl₂ at varying concentrations. In our first preliminary experiment, 8 fish (2 fish per tank) were exposed to 0, 1, 2 and 25 p.p.b. Cd in 300 l. of aerated fresh water for 24 h. Uncontaminated fresh water in a continuous flow system was then run into the tanks, and the fish killed on day 7. Only the testis of the fish exposed to 25 p.p.b. Cd for 24 h showed marked discoloration. Dark purple-brown patches were scattered randomly throughout the testes, in contrast to the characteristic creamy-white testes of the control fish. In the second experiment, 36 brook trout were exposed to 10 p.p.b. Cd in a freshwater continuous flow system and the LT₅₀ was found to be 21 days. (During the experiment, the freshwater temperature ranged from 8° C to 11° C.) Immediate post-mortem examination was possible in only 8 male Cd-treated fish, and of these 7 had abnormally vascularized discoloured testes. The testes of the corresponding 20 male control fish were not damaged. The testes of fish from both experimental groups were either in the later pre-spawning phase (stage 4) or fully mature (stage 5)⁷.

Histological examination of the damaged and control tissues revealed marked differences (Figs. 1 and 2). The testes of the 25 p.p.b. Cd-treated fish revealed extensive haemorrhagic necrosis (Fig. 2). Many blood vessels, packed with erythrocytes and greatly distended, were visible between the testes' lobules. The blood vessel walls had collapsed in places and many erythrocytes had surrounded or invaded the lobules, causing the lobule-boundary cells to disintegrate and the nuclei to become pyknotic. In the second experiment the damaged testes of the 10 p.p.b. Cd-exposed fish showed similar injury, but the damage was not so extensive. In both experimental groups there was no damage to the primordial germ cells, which in the absence of a germinal epithelium in fish are the initial sites of spermatogenesis. This is the first report of Cd-

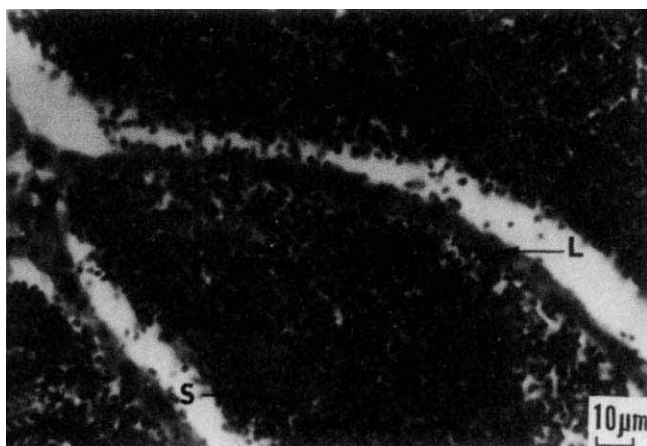


Fig. 1 Normal mature testis (stage 5) of control fish. Testis lobule is full of ripe spermatozoa (S), enclosed by lobule-boundary cells (L) (haematoxylin and eosin).

induced testicular injury in fish. It is interesting that a poikilotherm, possessing abdominal testes with relatively "simple" vasculature, and lacking a germinal epithelium, can also be adversely affected by Cd.

Histochemical investigation of the lipid distribution of control and damaged testes, using sudan black B, revealed accumulation of lipids in the lobule-boundary cells only, indicating these are the probable sites of steroid production (the Leydig cells) in this species. In the damaged testes a very weak sudanophil staining reaction was observed in the lobule-boundary cells, indicating a reduction of lipid material which provides some evidence that androgen production has been disturbed. There was no regeneration of Leydig cells in the damaged testes.

11-Ketotestosterone (11 KT), 11 β -hydroxytestosterone (11 OHT) and testosterone (T) provided the logical index for studying the effects of Cd on testicular androgenesis in the brook trout since these steroids are potent androgens for some fish⁸. In the Atlantic salmon *Salmo salar* there was a positive correlation between the levels of 11 KT, 11 OHT, and T and increasing sexual maturity⁹. *In vitro* incubations with fish testes also indicated 11 OHT and T are potential precursors of 11 KT⁸.

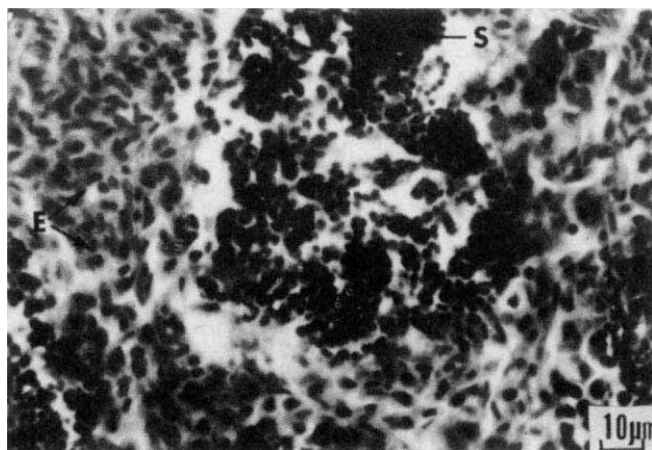


Fig. 2 Cadmium-damaged testis (stage 5) of fish exposed to 25 p.p.b. Cd. Extensive haemorrhagic necrosis is visible with large numbers of erythrocytes (E) surrounding and invading the testis lobule. The lobule-boundary cells have disintegrated and the dark-staining spermatozoa (S) are left in the centre of the lobule (haematoxylin and eosin).

When equal amounts (500 mg) of tissue from the mid-testes of the fish exposed to 25 p.p.b. Cd and the corresponding control were each incubated with 0.5 μ Ci of 4-¹⁴C-pregnenolone (52.8 mCi/mmol) for 4.5 h at 8–10° C in medium containing NADPH-generating co-factors¹⁰, biosynthesized ¹⁴C-labelled 11 KT was detectable in the control but not in the Cd-damaged tissue incubate. (The recovery of 11 KT was 77.8% for the control and 76.6% for the Cd-incubate.) The yields of 11 OHT and T were significantly higher in the control than in the Cd-incubate. There was ~24% less conversion of radioactive pregnenolone to dichloromethane-extractable metabolites—"free" or unconjugated steroids—in the Cd-incubate than in the control. Both control and damaged testes were in the same stage of maturation but their histological pictures differed vastly. It is possible that the failure to detect 11 KT in the Cd-incubate may be correlated with the weak lipid-staining reaction in the lobule boundary cells of the damaged testes.

We then investigated the effects of varying concentrations of Cd on steroid synthesis by normal mature testes of brook trout *in vitro*. Four 1-g samples from pooled minces of mid-testes were each incubated as above but with 1.0 μ Ci 4-¹⁴C-pregnenolone in 10 ml. incubation medium to which was added 0, 10, 100 and 1,000 μ g Cd (as CdCl₂), respectively. A fraction

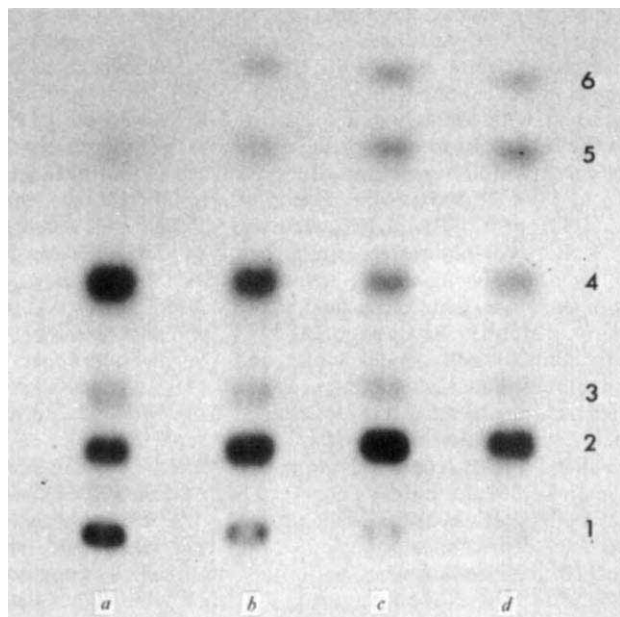


Fig. 3 Autoradiogram of the dichloromethane extracts of normal testes of *S. fontinalis* incubated with $4\text{-}^{14}\text{C}$ -pregnenolone in the presence of 0–1,000 μg Cd/g tissue *in vitro*. a to d represent 1/10 of 1 g samples incubated with 0, 10, 100 and 1,000 μg Cd respectively. 1 to 6 indicate the positions of radioactive areas. Areas 1 and 3 indicate the position of 2 unknown products; areas 2, 4, 5 and 6 were isopolar with radioinert carrier steroids 11 OHT, 11 KT, T, and pregnenolone, respectively.

of the dichloromethane extract of each incubate was chromatographed on a thin-layer plate developed in one direction in two solvent systems. X-ray autoradiography of the chromatogram indicated marked differences between the control (+0 μg Cd) and the Cd-treated incubates (Fig. 3). The radioactive profile showed that Cd also altered the biosynthesis of unknown metabolites not included in our analyses; that is, areas 1 and 3 and also areas 2, 4 and 5 which were isopolar with 11 OHT, 11 KT and T, respectively, but only fractions were actually associated with the corresponding biosynthesized ^{14}C -labelled steroids.

Analysis of each testicular incubate gave the following significant results. First, Cd inhibited the conversion of exogenous pregnenolone to free steroids; the amount of dichloromethane-extractable radioactivity decreased as the concentration of added Cd increased. Second, Cd inhibited the biosynthesis of 11 KT from $4\text{-}^{14}\text{C}$ -pregnenolone. The product yield ratios of 11 KT/T decreased progressively 1.22, 0.46, 0.23 and 0.15 and those of 11 OHT/11 KT increased 3.22, 8.55, 18.2 and 14.1 at 0, 10, 100 and 1,000 μg Cd/g incubated tissue, respectively. With no Cd added and at less than 1,000 μg Cd the ratios of 11 OHT/T remained practically constant at 3.94–4.20. Since 11 KT synthesis was already appreciably reduced at the lower levels of Cd we speculate on the possibility of 11 KT being synthesized via a different route bypassing 11 OHT and T and/or 11-dehydrogenation of 11 OHT to 11 KT may be directly inhibited by Cd. Third, at 1,000 μg Cd/g incubated tissue there was a general reduction in the product yields of 11 KT, 11 OHT and T accompanied by a decrease in the ratios 11 KT/T, 11 OHT/11 KT and 11 OHT/T. Identification of each labelled steroid was based on the isopolarity and isomorphism of the product and/or its acetate derivative with the corresponding authentic ^3H -labelled and radioinert reference steroids^{11,12}.

Our experimental results give conclusive evidence that Cd can directly affect steroid enzyme systems *in vitro*. Whether the results reflect the situation *in vivo* is not known. The implications of reduced 11 KT synthesis in fish testes *in vitro* cannot be ignored. There is little attention drawn to the effects of apparently non-lethal levels of pollutants on the reproductive

physiology of fish and other aquatic life. Yet the most serious threat of pollution in our environment could well be its adverse but subtle effects on the capacity of species to reproduce. A full report including all experimental details will be published elsewhere.

We thank Dr E. G. Bligh and Mr H. C. Freeman for encouragement.

G. B. SANGALANG
M. J. O'HALLORAN

Fisheries Research Board of Canada,
Halifax Laboratory,
PO Box 429,
Halifax, Nova Scotia

Received May 19; revised August 11, 1972.

- ¹ Parizek, J., *J. Reprod. Fert.*, **1**, 294 (1960).
- ² Gunn, S. A., and Gould, T. C., in *The Testis* (edit. by Johnson, A. D., Gomes, W. R., and Vandemark, N. L.), **3**, 411 (Academic Press, New York, 1970).
- ³ Kar, A. B., and Das, R. P., *Acta Biol. Med. Ger.*, **5**, 155 (1960).
- ⁴ Pauffer, S. K., and Foote, R. H., *J. Reprod. Fert.*, **19**, 309 (1969).
- ⁵ Favino, A., Baillie, A. H., and Griffiths, K., *J. Endocrinol.*, **35**, 185 (1966).
- ⁶ Gomes, W. R., in *The Testis* (edit. by Johnson, A. D., Gomes, W. R., and Vandemark, N. L.), **3**, 95 (Academic Press, New York, 1970).
- ⁷ Henderson, N. E., *Can. J. Zool.*, **40**, 631 (1962).
- ⁸ Idler, D. R., Truscott, B., and Stewart, H. C., in *Progress in Endocrinology* (edit. by Gual, C.), 724 (Excerpta Medica Foundation, Amsterdam, 1969).
- ⁹ Idler, D. R., Horne, D. A., and Sangalang, G. B., *Gen. Comp. Endocrinol.*, **16**, 257 (1971).
- ¹⁰ Idler, D. R., and Sangalang, G. B., *J. Endocrinol.*, **48**, 627 (1970).
- ¹¹ Idler, D. R., and Macnab, H. C., *Can. J. Biochem.*, **45**, 581 (1967).
- ¹² Axelrod, L. P., Matthijssen, C., Goldzieher, S. W., and Pulliam, J. E., *Acta Endocrinol.* (Copenhagen), Suppl., **49**, 99 (1965).

Can "Blocking" Serum Factors Protect against Autoimmunity?

HUMANS and laboratory animals carrying progressively growing tumours possess, as a rule, lymphocytes capable of specifically destroying their own neoplastic cells *in vitro*¹. Sera from the tumour-bearers can specifically block that destructive effect². The nature of the blocking factors is unknown, but there is some evidence that they are complexes between antigen released by the tumour and antibodies formed by the host³. They can be removed from serum by absorption with the appropriate tumour cell type³.

The coexistence of specifically cytotoxic lymphocytes and blocking serum factors has also been detected in some conditions not involving neoplasia². Mice which have undergone pregnancy with allogeneic males have lymphocytes capable of destroying cells carrying the paternal antigens, and sera which will block that effect⁴; irradiated dogs, mice and immunodeficient children, permanently repopulated with allogeneic bone marrow and not showing graft versus host reactivity, have been found to have lymphocytes (of donor type) capable of destroying cultivated fibroblasts (of host type)^{5–7}; blocking serum factors, as well as specifically cytotoxic lymphocytes, have been detected in mice and rats rendered tolerant to allografts (ref. 8 and our unpublished work with S. C. Bansal and H. O. Sjögren) and in tetraparental mice^{9,10}.

A crucial question is, of course, whether a blocking serum effect, similar to that seen in tumour-bearing animals and in cases of allograft tolerance, plays any role for the establishment and maintenance of tolerance to normal tissue specific antigens. Indeed, there are cases in which tolerance to such "self" antigens (for example, to serum proteins) does not seem to be due to any blocking serum activity but may be best explained as the result of elimination of those lymphoid clones which

would have been genetically capable of reacting¹¹. In other conditions, however, a blocking serum activity, coexistent with cytotoxic lymphocytes, may be involved. We have searched for the latter type of situation. The experiments reported here have been based on the assumption that blocking serum factors, if they exist, may be removed by absorption with cells containing the appropriate type of tissue specific antigens, and that syngeneic lymphocytes may be cytotoxic to normal cells in the absence of the specific blocking factors.

Cell cultures were established from brains and kidneys of newborn (A × C3H)F₁ and C57BL mice, using standard techniques involving trypsinization of the tissue. The cultures were maintained in Eagle's minimum essential culture medium (MEM) supplemented with 20% foetal calf serum. Brain cultures consisting of spindle shaped nerve-like cells were selected for the study, as were those kidney cultures which were primarily made up of epithelial cells (no studies using specific markers such as acetylcholinesterase for nerve cells have been conducted yet). While it was relatively easy to establish brain cultures which consisted of a larger fraction of nerve-like cells than of fibroblastic ones, it was never possible to get more than about 50% of the cells in a kidney culture with epithelial morphology, and in most trials the kidney cultures predominantly consisted of fibroblasts.

Lymph node cells (LNC) were collected from axillary, cervical and inguinal lymph nodes of 2–3-month-old syngeneic (A × C3H or C57BL) mice. They were brought into suspension by being pressed through a 60-mesh stainless steel screen. Sera were collected from the same type of mice providing the LNC. Most sera were first absorbed (see below), but unabsorbed sera from all pools were also tested. All sera were heat inactivated at 56° C for 30 min before the tests.

In an attempt to remove hypothetical target cell specific blocking factors, mouse sera were absorbed by incubation for 20 min at room temperature with monolayers of growing brain or kidney cells from the respective strain studied in a given experiment. The cultures used for absorption were grown in serum-free medium for 24 h beforehand. An 8 ml. sample of serum diluted 1 : 4 with phosphate-buffered saline was added to each 260 ml. disposable tissue culture flask (Greiner and Sons, Nürtingen, Germany), in which approximately 10⁷ cells were growing. The same absorption procedure was repeated two more times using fresh cells. All foetal calf sera used in our tests were absorbed three times with cultivated brain cells and three times with cultivated kidney cells, using the same procedure as for the mouse sera, except that the foetal calf serum was not diluted first.

The microcytotoxicity assay used before¹² was chosen to search for cellular immunity and blocking serum activity. Brain and kidney cells, cultivated during 2 weeks to 3 months, were trypsinized and seeded, 60–80 cells/well, into the wells of Falcon No. 3040 'Microtest' plates. MEM was used, to which 10% foetal calf serum absorbed with both brain and

kidney cells had been added. The following day, when the target cells had attached to the bottoms of the wells, the medium was removed and 1 : 4 diluted mouse serum added; groups were included in which the mouse serum had been absorbed with kidney or with brain cells, as well as groups which got unabsorbed mouse serum and groups which did not get any mouse serum at all. Following 45 min of incubation at 37° C, the sera were poured off and normal, syngeneic (A × C3H or C57BL) LNC were added, each well receiving 10⁵ LNC. The plates were gently rocked on a Belco rocker for 60 min and then foetal calf serum (absorbed with kidney and brain cells) was added to a final concentration of 10%. After approximately 36 h incubation at 37° C in 5% CO₂, the plates were stained with crystal violet and the mean number of remaining, attached target cells/well (± s.e.) calculated. Statistical significance was determined from Student's *t* tests. Each determination consisted of twelve replicates.

Table 1 presents one experiment in which nerve-like brain cells and epithelial kidney cells were both available. It shows that both A × C3H and C57BL normal syngeneic lymphocytes had a cytotoxic effect on those brain cells which had been exposed to serum absorbed with other brain cells as compared with brain cells exposed to serum absorbed with kidney cells; the effect was reversed when kidney cells were used as targets. The number of cells in wells exposed to unabsorbed serum was similar to that in wells to which serum absorbed with the control tissue (kidney with brain target cells and *vice versa*) had been added. In the absence of any exposure to serum, a cytotoxic lymphocyte effect was observed. If neither LNC nor serum was added, the number of surviving target cells was slightly lower than in the control groups with unabsorbed serum and LNC; for example, in experiment 6a (presented in Table 1) there was 15.3 ± 2.4 cells/well in the absence of both LNC and serum.

All the experiments performed are summarized in Table 2. It seems that results similar to those obtained in the one experiment (No. 6) listed in Table 1 were obtained in most tests with brain target cells (in nine of twelve cases). On the other hand, only three of nine experiments with kidney target cells showed a significant cell mediated immunity in groups exposed to serum absorbed with kidney, as compared with brain cells. This may be due to the fact that although nerve-like brain cells could be regularly cultivated, most of the kidney cultures contained a large fraction of fibroblastic rather than epithelial cells. Alternatively, the brain data may represent an exception and, for example, may be explained by the relative immunological separation of the brain from the rest of the organism. Since no significant stimulation was seen in any of the tests when kidney cells were exposed to LNC following serum absorbed with kidney, it is not likely that the effects observed with brain targets were just due to nonspecific toxicity of serum absorbed with brain cells. No significant differences in the number of surviving target cells per well were

Table 1 One Experiment (No. 6 in Table 2) in which Brain and Kidney Cells were First Exposed to Syngeneic Serum absorbed with Syngeneic Brain or Kidney Cells and then to Lymph Node Cells from Untreated Syngeneic Donors

Experiment	Target cells		Serum absorbed with				Unabsorbed serum		No serum	
	Strain	Tissue of origin	Brain cells Cells/well*	% Red †	Kidney cells Cells/well*	% Red †	Cells/well*		Cells/well*	% Red ‡
6a	C57BL	Brain	12.3 ± 1.3	33.2 ‡	18.3 ± 2.6	Contr.	19.8 ± 2.2		10.4 ± 1.0	47.5 §
6b	C57BL	Kidney	14.0 ± 1.7	Contr.	13.2 ± 1.9	6.1	12.8 ± 1.3		8.4 ± 0.9	34.4 §
6c	A × C3H	Brain	18.1 ± 1.4	32.8 ‡	26.8 ± 3.8	Contr.	29.3 ± 2.0		20.9 ± 2.8	28.7 ‡
6d	A × C3H	Kidney	26.8 ± 2.5	Contr.	20.0 ± 1.5	25.4 ‡	35.3 ± 3.4		20.5 ± 2.7	41.9 §

* Means ± s.e. are given.

† Percentage reduction was calculated by comparing the numbers of remaining attached target cells in wells given experimental group serum (=absorbed with the same type of cells as the targets) with those in wells given control serum (=absorbed with cells different from the targets). Probabilities that differences are due to chance: ‡ *P* 0.05, § *P* 0.01.

‡ Percentage reduction was calculated by comparison with wells exposed to unabsorbed syngeneic serum before they received the same number of syngeneic LNC as the wells to which no serum had been added.

Table 2 Summary of Experiments in which Cultivated Brain and Kidney Cells were First Exposed to Syngeneic Serum absorbed with Cultivated Syngeneic Brain or Kidney Cells and then to Lymph Node Cells (LNC) from Unsensitized Syngeneic Donors

Experiment	Target cells Strain Tissue of origin	Percentage reduction *		
		Abs. with serum	Abs. with kidney cells	Without exposure to serum
1a	A × C3H Brain	29.0 †	Contr.	26.1 †
1b	A × C3H Kidney	Contr.	18.6	48.9 §
2	C57BL Brain	22.9 †	Contr.	32.1 †
3	A × C3H Brain	12.6 †	Contr.	60.3 †
4a	A × C3H Brain	-3.4	Contr.	17.8 §
4b	A × C3H Kidney	Contr.	20.5 †	18.7
4c	C57BL Brain	24.1 †	Contr.	23.6 §
5a	C57BL Brain	45.2 †	Contr.	35.0 †
5b	C57BL Kidney	Contr.	-15.5	18.6 †
5c	A × C3H Brain	16.4 †	Contr.	22.2 †
5d	A × C3H Kidney	Contr.	-15.9	17.7 †
6a	C57BL Brain	33.2 †	Contr.	47.5 †
6b	C57BL Kidney	Contr.	6.1	34.4 †
6c	A × C3H Brain	32.8 †	Contr.	28.7 †
6d	A × C3H Kidney	Contr.	25.4 †	41.9 †
7a	A × C3H Brain	28.4 †	Contr.	18.8
7b	A × C3H Kidney	Contr.	-1.5	12.8 †
8a	A × C3H Brain	2.1	Contr.	29.4 §
8b	A × C3H Kidney	Contr.	14.4	33.5 §
9a	A × C3H Brain	12.5	Contr.	29.2 †
9b	A × C3H Kidney	Contr.	25.5 †	26.6 †

In experiment No. 9a and b, each well received 2×10^5 LNC/well as compared to 10^5 LNC/well in all the other experiments.

* Probabilities that differences are due to chance: † $P < 0.05$, ‡ $P < 0.01$, § $P < 0.001$.

|| Percentage reduction was calculated by comparing the numbers of remaining attached target cells in wells given experimental group serum (=absorbed with the same type of cells as the targets) with those in wells given control serum (=absorbed with cells different from the targets); the latter figure is by definition 0% reduction and given as control (contr.).

¶ Percentage reduction was calculated by comparing wells exposed to unabsorbed syngeneic serum before they received the same number of syngeneic LNC as the wells to which no serum had been added.

seen in groups incubated with serum absorbed with either brain or kidney cells but not subsequently exposed to LNC; this indicates that absorption with brain (kidney) cells does not release any factor cytotoxic to brain (kidney) cultures.

The data presented are possibly analogous to the findings of Cohen *et al.*¹³, according to whom lymphocytes *in vitro* can acquire the capacity to react against normal cultivated syngeneic fibroblasts. Some recent findings of Pierce may also be related to our observations: he reported that parental strain LNC have the capacity to destroy cultivated normal (A × C3H) F₁ fibroblasts, and that this capacity can be abrogated by exposing the target cells to normal A × C3H serum¹⁴.

One explanation of our findings, which we like to consider now, is that the mice studied could react against antigens present on their normal (brain) cells by mounting a cell-mediated immunity against them, and that they had blocking serum factors, removable by absorption with the respective tissues. The blocking factors would then provide a mechanism for avoiding *in vivo* tissue destruction by specifically cytotoxic lymphocytes; since they could be absorbed with target cells, the blocking factors are more likely to have been antigen-antibody complexes (or just antibodies) rather than antigens (but this does not exclude that antigen alone can play a role as blocker). Alternative explanations of our data must be kept in mind, however, including more trivial ones: the reactivity observed did not need to have been directed against a cell or tissue specific antigen but could, for example, have been directed against the antigen of a contaminating virus (or determined by such a virus), or have been directed against an antigen present on the cells studied as a result of these having been explanted *in vitro*.

The possible role of the phenomena described for the establishment and maintenance of "self" tolerance *in vivo* is unknown.

One way of approaching this question may be by studying the *in vivo* role of similar findings in the allograft tolerance situation, using mice and rats rendered neonatally tolerant by exposure to antigenic cells, as well as tetraparental (allophenic) mice. If studies performed in these systems would establish an important *in vivo* role of the cell-mediated immunity and blocking serum activity that can be detected *in vitro*—and we do not know if this will be the case—then there would be adequate cause to postulate that the reactivity presently described is relevant to the clinical phenomenon of auto-immunity. Such an *in vivo* role of cell-mediated immunity and blocking serum activity, detectable *in vitro*, has been demonstrated in tumour systems^{2,15}.

We thank Mrs Judi Bamer and Linda Katzenberger for technical assistance. This work was supported by grants and a contract from the National Institutes of Health and by a grant from the American Cancer Society.

INGEGGERD HELLSTRÖM

KARL ERIK HELLSTRÖM

Departments of Microbiology and Pathology,
University of Washington Medical School,
Seattle, Washington 98195

Received August 18; revised October 2, 1972.

- Hellström, K. E., and Hellström, I., *Adv. Cancer Res.*, **12**, 167 (1969).
- Hellström, K. E., and Hellström, I., *Ann. Rev. Microbiol.*, **24**, 373 (1970).
- Sjögren, H. O., Hellström, I., Bansal, S. C., and Hellström, K. E., *Proc. US Nat. Acad. Sci.*, **68**, 1372 (1971).
- Hellström, K. E., Hellström, I., and Brawn, R. J., *Nature*, **224**, 914 (1969).
- Hellström, I., Hellström, K. E., Storb, R., and Thomas, E. D., *Proc. US Nat. Acad. Sci.*, **66**, 65 (1970).
- Hellström, I., Hellström, K. E., and Trentin, J. J., *Cellular Immunol.* (in the press).
- Jose, D. G., Hersey, J. G., Choi, J. S., Biggar, W. D., Gatti, R. A., and Good, R. A., *Lancet*, **ii**, 841 (1971).
- Hellström, I., Hellström, K. E., and Allison, A. C., *Nature*, **230**, 49 (1971).
- Wegmann, T. G., Hellström, I., and Hellström, K. E., *Proc. US Nat. Acad. Sci.*, **68**, 1644 (1971).
- Phillips, S. M., Martin, W. J., Shaw, A. R., and Wegmann, T. G., *Nature*, **234**, 146 (1971).
- Dresser, D. W., and Mitchison, N. A., *Adv. Immunol.*, **8**, 129 (1968).
- Hellström, I., and Hellström, K. E., in *In vitro Methods in Cell-Mediated Immunity* (edit. by Bloom, B. R., and Glade, P. R.), 409 (Academic Press, New York and London, 1971).
- Cohen, I. R., Globerson, A., and Feldman, M., *J. Exp. Med.*, **133**, 834 (1971).
- Pierce, G. E., *Cellular Immunol.*, **3**, 150 (1972).
- Sjögren, H. O., and Bansal, S. C., in *Progress in Immunology* (edit. by Amos, B.), 921 (1971).

Possible Facilitated Transport of Oxygen across the Placenta

THE function of the placenta requires that some substances pass easily while others do not. There are specific transport mechanisms for essential sugars whereas permeability to most ions and unionized solutes is small¹⁻³.

We have calculated the permeability of the maternal foetal exchange capillary to inert gases and water⁴. The permeability coefficient, K , with units of ml./min relates flux to the driving gradient of concentration in Fick's first law $J = K \Delta C$. J is the flux in ml./min and ΔC is the difference in concentration in ml. dissolved gas/ml. fluid. We found that values of K , for the maternal foetal exchange capillary, from 200–260 ml./min fit our data. The diffusing capacity of the sheep placenta to

CO is approximately $\frac{1.25 \text{ ml./min}}{\text{mm Hg}}$, while O₂ diffusing capacity

is thought to be 1.2 to 2 times the CO diffusing capacity⁵. We can express our permeability coefficients as diffusing capa-

city by dividing by the partial pressure necessary to dissolve 1 ml. of gas in 1 ml. of fluid. For argon, which has physical properties similar to O₂ and CO, the diffusing capacity for the whole placenta is 0.01 $\frac{\text{ml./min}}{\text{mm Hg}}$ (this assumes that $K=200$

for the whole placenta, and a bunsen solubility coefficient of 0.04 ml./760 mm Hg). Thus, there seem to be two orders of magnitude difference in the ease with which CO and O₂ can cross the placental barrier compared with argon.

Longmuir⁶ has shown that the relationship between O₂ uptake and surface PO₂ in liver slices is described by Michaelis-Menton kinetics, rather than as predicted by simple diffusion models. He suggested that O₂ transfer (in the liver) is partially carrier-mediated and that the carrier is a microsomal cytochrome (P-450) involved in the metabolism of hormones and other endogenous and exogenous compounds. This cytochrome is also present in the placenta in microsomes and in mitochondria⁷. Our results may have been due to the P-450 facilitation of O₂ transport.

Several drugs bind to cytochrome P-450 and are metabolized by enzyme systems associated with the cytochrome. Each competitively inhibits the metabolism of the others, and the site of competition has been set at the level of the oxygen-carrying cytochrome P-450 (ref. 8). The function of this cytochrome requires that it be bound to a lipid membrane. Emulsifying agents used to solubilize the enzyme have been found to change it to cytochrome P-420, an inactive form⁹.

We report here the effect on placental O₂ transfer of the drugs SKF-525A (2-diethyl-amino-ethyl-2,2-diphenyl valerate), analine, morphine, and meperidine, all of which bind to cytochrome P-450, and an emulsifying agent (Na⁺ deoxycholate), known to inactivate P-450 *in vitro*.

It has been reported that women addicted to morphine-type drugs produce abnormally small offspring¹⁰. We have investigated the effect of these drugs on placental O₂ transfer since we thought the failure of the foetus to attain normal size may be due in part to interference with transplacental O₂

flux. In all experiments argon transfer was measured simultaneously. If the drugs caused any non-specific changes in the placenta, argon transfer would be affected as well as oxygen.

Since the metabolism of drugs by means of the cytochrome P-450 systems involves the addition of an oxygen molecule to the compound, any decrease in transplacental flux of O₂ might be due to increased O₂ utilization by the placenta. To attempt to separate the metabolic effect from an effect on the rate of O₂ transfer, we measured the utero-placental oxygen consumption before and after administration of the various compounds.

The *in vivo* perfusion technique has been described in detail before. Briefly, in term ewes the uterus was opened, the umbilical cord severed and the whole placenta perfused from the foetal side using a roller pump with a dextran/saline solution or with blood from an adult sheep which had been equilibrated with argon at 37° C. Partial pressures of oxygen and argon were measured in umbilical artery and vein and in maternal artery and uterine vein using a membrane-tipped catheter connected to a mass spectrometer⁴. The test compounds were dissolved in perfusion fluid and infused as a slug into the umbilical artery during steady state gas exchange.

Since the amount of argon lost by the foetal circulation is gained by the maternal circulation, it is possible in a steady state to calculate maternal utero-placental blood flow by the Fick principle if foetal perfusion rate is known and the partial pressure of argon is measured in umbilical vein, umbilical artery, maternal artery and uterine vein. If the O₂ content of maternal uterine venous and arterial blood is also known, utero-placental O₂ consumption¹¹ can be calculated. We measured utero-placental O₂ consumption before and after administration of drugs.

In experiment 1 when 1 g of SKF-525A was injected into the umbilical artery, O₂ transfer declined sharply while inert gas transfer did not change markedly. Fig. 1 shows the P_{O₂} and P_{argon} of perfusion fluid leaving the placenta before,

Table 1 Effects of Drugs on Simultaneous Placental Oxygen and Argon Transfer

Experiment	Drug and dose	Side of delivery of drug	Perfusion fluid	% Change in transplacental O ₂ flux	% Change in transplacental argon flux	Utero-placental O ₂ uptake ml./min.	
						Before drug	After drug
1	SKF-525A 1 g	F	D	-78.5	+20	—	—
2	SKF-525A 0.5 g	F	D	-78	-9	—	—
3	SKF-525A 1 g	F	D	-88	-12.5	15	13.5
4	SKF-525A 0.5 g	M	D	-70	+7.5	13.5	11
5	Analine 2 g	F	D	-85	+3	17	13
6	Analine 1.5 g	M	D	-75	-2	19	17
7	Na-deoxy cholate 0.3 g	F	D	-78	-7	16	14
8a	Metapyrone 0.7 g	F	D	-37	0	13	13
8b	SKF-525A 0.2 g	F	D	-66	-6	13	16
9a	Morphine 30 mg	After metapyrone F	D	-20	0	—	—
9b	Meperidine 100 mg	F	D	-60	0	—	—
9c	SKF-525A 0.3 g	After morphine F	D	-84	-15	—	—
10	SKF-525A 0.7 g	After meperidine M	D	-70	0	14	11
11	SKF-525A 1.4 g	F	B	-77	0	14*	13.75*
12	SKF-525A 1.5 g	F	B	-70	+5	13*	12.5*

F, foetal circulation; M, maternal circulation; D, dextran solution; B, blood.

* These O₂ uptakes do not include transplacental flux of O₂.

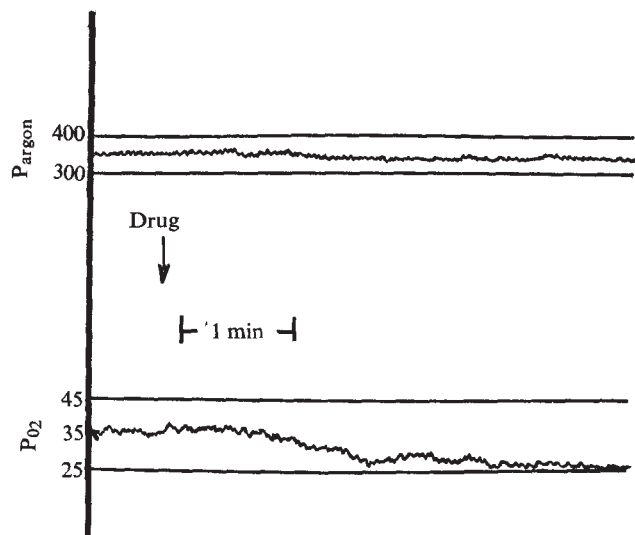


Fig. 1 Simultaneous measurement of P_{argon} and P_{O_2} in the umbilical vein. Before infusion of the drug gas exchange had been in a steady state. About 1 min after slug injection of SKF-525A (a compound which binds to cytochrome P-450), P_{O_2} decreased markedly, indicating a decrease in transplacental O_2 flux.

during and after infusion of SKF-525A. Table 1 gives the results of all the experiments: the percentage change in flux of O_2 and argon is given before and after the various drugs.

$$\% \text{ change} = \frac{(\text{Puv-Pua}) \text{ after drug} - (\text{Puv-Pua}) \text{ before drug}}{(\text{Puv-Pua}) \text{ before drug}}$$

In the case in which the placenta was perfused with blood (experiments 11 and 12) the transplacental O_2 flux was calculated from the O_2 content of blood from the foetal umbilical artery and vein. In these two experiments the transplacental O_2 flux was 6 ml. and 10 ml./min before the drug and 1.2 and 2 ml./min after the drug.

Oxygen flux decreased markedly after administration of each test compound while argon flux did not change systematically. Utero-placental O_2 consumption generally decreased or remained constant after administration of the drugs. In only one case did O_2 consumption appear to increase. The values of utero-placental O_2 consumption agree well with those measured by Meschia *et al.*¹¹ for sheep.

The difference between argon and O_2 diffusing capacities indicates that the effective permeability of the foetal-maternal exchange capillaries is greater to O_2 than to argon. It is difficult to explain this large permeability to O_2 on a physical basis, since its physical properties such as water solubility, oil-water partition coefficient and diffusion coefficient, are fairly similar to those of argon. This evidence suggests a specific transport mechanism in the placenta for O_2 .

Longmuir's experiments suggest that the carrier is cytochrome P-450. We reasoned that drugs which bind to cytochrome P-450 decrease the ability of this compound to act as an O_2 carrier. We observed that drugs which bind to the cytochrome decreased O_2 transfer without increasing the O_2 uptake of the placenta or affecting inert gas transfer. Na^+ deoxycholate which inactivates cytochrome P-450 *in vitro* also decreases O_2 transfer. Although there is no information to indicate that this compound acts in a similar manner *in vivo*, we have no reason to suppose that it should act differently.

This sort of correlation between the ability to bind to or inactivate the cytochrome and the ability to decrease O_2 transfer seems to support the hypothesis that cytochrome P-450 acts physiologically as an O_2 carrier in the placenta as well as acting in metabolism of drugs and in hormone synthesis.

If the drugs interfered non-specifically with gas exchange they should have caused a decrease in argon transfer as well as O_2 transfer, which they did not. If the drugs decreased O_2

transfer by increasing O_2 utilization by the placenta we should have been able to demonstrate an increase in utero-placental O_2 consumption after the drugs. We observed either no increase or a decrease in O_2 consumption in all but one experiment.

Morphine and meperidine also seem to decrease transplacental O_2 transfer. We suggest that interference of the drug with transplacental O_2 transfer contributes to the failure of the foetus to attain normal size.

We thank Dr Robert J. Rubin for advice and Smith, Kline and French Laboratories for SKF-525A.

G. H. GURTNER
B. BURNS

Department of Environmental Medicine,
Johns Hopkins University,
School of Hygiene and Public Health,
615 North Wolfe Street,
Baltimore, Maryland 21205

Received June 16; revised August 15, 1972.

- ¹ Widdas, W. F., *J. Physiol.*, **118**, 23 (1952).
- ² Nixon, D. A., *J. Physiol.*, **166**, 351 (1963).
- ³ Meschia, G., Battaglia, E., and Barron, D., *J. Appl. Physiol.*, **22**, 1171 (1967).
- ⁴ Bissonnette, J. M., and Gurtner, G. H., *J. Appl. Physiol.*, **32** (1), 64 (1972).
- ⁵ Longo, L. D., Power, G. G., and Forster, R. E., *J. Clin. Invest.*, **46**, 812 (1967).
- ⁶ Longmuir, I. S., in *Microcirculatory Approaches to Current Therapeutic Problems*, Symposia, Sixth Europ. Conf. Microcirculation, Aalborg, 1970, 3 (Karger, Basel, 1971).
- ⁷ Meigs, R. A., and Ryan, K. I., *Biochim. Biophys. Acta*, **165**, 476 (1968).
- ⁸ Mannering, G. J., in *Selected Pharmacologic Testing Methods* (edit. by Berger, A.) (Dekker, New York, 1968).
- ⁹ Imai, Y., and Sato, R., *Europ. J. Biochem.*, **1**, 419 (1967).
- ¹⁰ Zelson, C., *Pediatrics*, **48**, 178 (1971).
- ¹¹ Meschia, G., Cotter, J., Markowski, E., and Barron, P., *Quart. J. Exp. Physiol.*, **52**, 1 (1966).

Influence of the Embryo on the Marsupial Uterus

I REPORT here evidence of a local morphogenetic action by the marsupial embryo or placenta on the uterus. The marsupial placenta is usually said not to be an endocrine organ and to have no influence on uterine changes during pregnancy. This assumption is based on the failure of pregnancy to extend the life of the active corpus luteum or to interrupt the oestrous cycle^{1,2}. The gravid uterus of macropodid marsupials becomes larger than the contralateral, non-pregnant uterus, but this has been attributed to the bulk of the embryo occupying it, although observations by Flynn³ suggest otherwise. Evidence is given below that the two uterine horns of the wallaby, *Macropus eugenii*, are distinct in a number of ways, and that it is the presence or absence of the embryo which determines the differences.

This species is monovular and polyoestrous. Ovulation occurs in alternate ovaries during successive oestrous cycles, so that only one of the two uterine horns becomes pregnant. Animals were autopsied at various stages of gestation by a procedure already described⁴. Each uterus was cut longitudinally; the endometrium was completely stripped from the myometrium, weighed, frozen and stored at -20°C .

Endometria from both uteri of non-pregnant, cycling animals became heavier during the development of the luteal phase. Weights of both endometria declined at pro-oestrus, and returned to quiescent levels. The endometrium from the uterus on the side with an active corpus luteum was always heavier than that on the other side ($n=24$, $P<0.05$, Wilcoxon matched pairs test). This suggests an action of the corpus

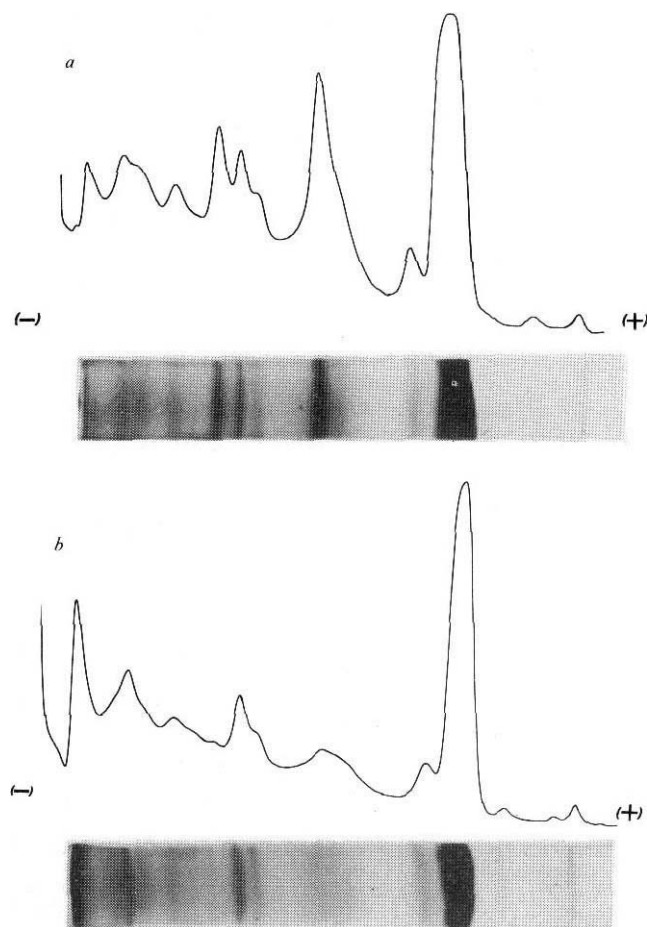


Fig. 1 Disc electrophoretic patterns of endometrial exudates from (a) pregnant and (b) non-pregnant uterine horns of the same animal taken at day 24 of gestation. Spectrophotometric scans of the gels at 625 m μ are also given. The uterine-specific β -globulin was virtually absent from the non-pregnant exudate, although it appeared as one of the major bands in the pregnant exudates. This pattern was observed in all exudates collected at day 13 or later.

luteum similar to that described in the brush possum, *Trichosurus vulpecula*⁵.

Table 1 gives the wet weights of endometria from pregnant animals. The endometrium from the pregnant uterus was always heavier ($P < 0.05$, paired comparisons t -test) than that from the non-pregnant side, despite much individual variation. This finding suggests an effect of the embryo on the endometrium. The proximity of the active corpus luteum could, however, be responsible for the increase in weight. If the increase was due to the embryo or placenta, a transferred blastocyst should induce a similar change in a previously non-pregnant uterus. This was tested by transferring blastocysts to both uteri of non-mated, cycling recipients by the technique of Tyndale-Biscoe⁶. Eleven animals were used for double transfers (Table 2). Of these, six carried embryos at autopsy. In three, both uteri were pregnant. In one, the embryo grew in the uterus contralateral to the corpus luteum, and in the remaining two

the embryos grew in the ipsilateral uterus. All the uteri which contained embryos had endometria of weights similar to those of pregnant uteri of normal gestation; the non-pregnant uteri in which the transferred blastocyst failed to grow did not increase any more than the non-pregnant uteri of normal pregnancies. The remaining 5 animals were not pregnant in either uterus at autopsy, although all had active corpora lutea: in them both uteri were similar in weight to those of non-pregnant animals.

Table 2 Wet Weights (mg) of Endometria from Uteri containing Transferred Blastocysts

	Day of gestation	Endometria	
		Ipsilateral to corpus luteum	Contralateral to corpus luteum
Double pregnancies	20	554	442
	20	398*	816
	24	430	454
Single pregnancy in contralateral uterus	25	137	334
Single pregnancy in ipsilateral uterus	25	517	87
	25	620	72
Non-pregnant	20	53	32
	24	112	101
	25	150	70
	25	94	87
	26	66	48

* Contained a degenerating embryo at autopsy.

Samples of endometria were thawed, and the serous fluid that exuded from them was collected for protein analysis. Aliquots of the fluid were analysed by disc electrophoresis⁷. Protein content differed in several ways from that of serum; this suggested both active synthesis and selection by the uterine glands. β -Globulins increased in concentration in the pregnant uteri during gestation, in particular a single uterine-specific protein in the β -globulin region of the gel was found in large amounts in all samples tested at and after day 13 ($N=36$). A similar band was present only in low concentrations, or not at all, in exudates from the non-pregnant sides (Fig. 1), and in all samples collected at days 0 and 6 of gestation ($N=18$). Total volume of exudate and total protein content increased in exudates from pregnant uteri during gestation, but the uterine-specific β -globulin increased disproportionately to the others, suggesting that this is a qualitative as well as a quantitative increase in protein. The appearance of the uterine β -globulin coincided with the period of rapid expansion of the blastocyst and formation of the primitive streak. It is similar in electrophoretic mobility and time of appearance to "blastokinin"^{8,9} or "uteroglobin"^{10,11}, a protein isolated from the rabbit uterus which has growth-promoting properties. The protein in *M. eugenii*, like the increase in weight of the endometrium, is apparently the direct result of the embryo's presence: it was found only in pregnant uterine fluids, and was present in all pregnant uteri which carried transferred blastocysts, regardless of the side of the active corpus luteum.

The evidence, then, suggests that the embryo or placenta exerts a two-fold effect on the uterus. It exceeds the influence of the corpus luteum by increasing the weight of the endo-

Table 1 Mean Wet Weights (mg) $\pm$ s.e. of Endometria

	0	13	Stage of gestation (days)		24	25
			14 and 16	17 and 18		
Number of wallabies	23	5	6	12	15	4
Pregnant side	95 $\pm$ 11.6	378 $\pm$ 36.9	612 $\pm$ 41.6	511 $\pm$ 44.8	400 $\pm$ 25.4	249 $\pm$ 44.8
Non-pregnant side	71 $\pm$ 10.8	261 $\pm$ 33.8	331 $\pm$ 53.6	180 $\pm$ 20.9	58 $\pm$ 8.1	55 $\pm$ 16.0

metrium in the uterus which carries it; and it stimulates the production of not only more protein but also of uterine-specific proteins. The uterine changes may be due to a hormone produced by the embryo or its membranes, or to an immunological response. In either case, the embryo or placenta of a marsupial is shown for the first time to have important structural and biochemical effects on the uterus.

This study was supported by a grant from the Commonwealth Scientific and Industrial Research Organization to Dr C. H. Tyndale-Biscoe.

MARILYN B. RENFREE

Zoology Department,
Australian National University,
Canberra, ACT,
Australia

Received July 17, 1972.

- ¹ Sharman, G. B., *Science*, **167**, 1221 (1970).
- ² Tyndale-Biscoe, C. H., *J. Reprod. Fertil.*, **23**, 25 (1970).
- ³ Flynn, T. T., *Proc. Linn. Soc. NSW*, **55**, 506 (1930).
- ⁴ Renfree, M. B., *J. Reprod. Fertil.*, **22**, 483 (1970).
- ⁵ von der Borch, S. M., *J. Reprod. Fertil.*, **5**, 447 (1963).
- ⁶ Tyndale-Biscoe, C. H., *J. Reprod. Fertil.*, **6**, 41 (1963).
- ⁷ Davis, B. J., *Ann. NY Acad. Sci.*, **121**, 404 (1964).
- ⁸ Krishnan, R. S., and Daniel, J. C., *Science*, **158**, 490 (1967).
- ⁹ Daniel, J. C., and Krishnan, R. S., *J. Exp. Zool.*, **172**, 267 (1969).
- ¹⁰ Beier, H. M., *Biochim. Biophys. Acta*, **160**, 289 (1968).
- ¹¹ Beier, H. M., in *Ovo-Implantation. Human Gonadotrophins and Prolactin* (Karger, Basel and New York, 1970).

Release of Brain Dopamine as the Probable Mechanism for the Hypothermic Effect of D-Amphetamine

THE effects of D-amphetamine on the colonic temperature of rats depend on the ambient temperature¹. Rats kept in cold environments (4°–15° C, relative humidity 45%) exhibit profound hypothermia for at least 90 min after receiving D-amphetamine (7.5–15 mg/kg, given intraperitoneally). The same doses cause marked hyperthermia among rats kept at room temperature (20° C) or in a warm environment (37° C). The evidence we report here suggests that the D-amphetamine-induced hypothermia is mediated by the release of dopamine from brain neurones.

Male Sprague-Dawley rats (Charles River Laboratories), weighing 100–150 g, were housed six per cage at an ambient temperature of 22° C and relative humidity of 45%. They had access to food (Big Red Laboratory Animal Chow, Agway Inc., Syracuse, New York) and water *ad libitum*. Light ('Vita-Lite', Duro-Test Corp., North Bergen, New Jersey) was provided between 0090 h and 2100 h daily. Just before each experiment, rats were placed in individual cages; they were then injected with one of several drugs in 1.0 ml. of medium, and placed in an environmental chamber set at 4°, 20° or 37° C, and a relative humidity of 45%. Thirty minutes later, half of the rats in each experimental group received an intraperitoneal injection of D-amphetamine (15 mg/kg); the other animals received saline. Colonic temperature was measured by telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio) just before the first injection and at 10-min intervals thereafter. Each experimental group included at least twelve rats, and each study was repeated at least twice. All experiments were done between 1000 h and 1400 h.

As in previous experiments¹, the administration of D-amphetamine (15 mg/kg) to rats kept in an environment of 4° C caused significant hypothermia (Table 1). Animals receiving tyramine (50 mg/kg) or the amphetamine analogue β , β -difluoroamphetamine (15 mg/kg), two drugs that share the peripheral sympathomimetic effects of D-amphetamine but are much less able to enter the brain, failed to display significant hypothermia.

Table 1 Effects of Various Drugs on Colonic Temperature in Rats kept at 4° C

Drugs	Change in temperature (° C)
Saline	-0.6±0.3
D-Amphetamine	-2.6±0.6*
Tyramine	-0.8±0.4
β , β -Difluoroamphetamine	-0.6±0.6
Apomorphine	-3.3±0.5*
Clonidine	-6.8±0.9*
L-Dopa	-2.9±0.7*

Rats received the drug intraperitoneally and were immediately placed in an environmental chamber. The changes in temperature were determined by comparing data obtained at zero time and 30 min after treatment. Data in Tables 1 and 2 are given as mean $\pm$ s.d.

* $P < 0.001$ differs from animals receiving saline.

This suggested that the hypothermia results from a direct action of D-amphetamine on the brain.

Hypothermia was also observed among rats pretreated with drugs thought to increase central dopaminergic tone: apomorphine (10 mg/kg), clonidine (Catapres, 2 mg/kg), or L-dopa (100 mg/kg) (Table 1)^{2,3}. Moreover, the hypothermia following D-amphetamine was blocked in rats pretreated with pimozide (25 mg/kg), haloperidol (2 mg/kg), or diltan (JB-329, 4 mg/kg) (Table 2), drugs believed to block brain dopamine receptors^{3,4}.

Table 2 Effects of Various Drugs on D-Amphetamine-induced Hypothermia

Drugs	Change in temperature (° C)	
	Drug alone	Drug followed by D-amphetamine
Saline	0.5±0.4	-3.2±0.6*
Pimozide	-1.0±0.5	-1.5±0.4
Haloperidol	-2.0±0.4	1.5±0.6
Diltan	0.2±0.2	-0.3±0.5
6-Hydroxydopamine (intracis- ternal)+saline (i.p.)	-1.0±0.6	-0.7±0.7
Methysergide	-2.0±0.6	-4.2±0.7*
p-Chloramphetamine	-4.0±0.3	-7.1±0.8*
5,6-Dihydroxytryptamine (in- tracisternal)+saline (i.p.)	-1.3±0.4	-3.9±0.7*
6-Hydroxydopamine (i.p.)+ saline	0.6±0.3	-7.1±0.9*
Phenoxybenzamine	-5.0±0.9	-9.0±0.8*
Propranolol	-4.8±0.9	-7.3±0.9*

Animals received the drug and were immediately placed in an environmental chamber at 4° C. Thirty minutes later half of each group received D-amphetamine. The changes in colonic temperature were determined by comparing data obtained at zero time and 60 min after the first injection (that is, 30 min after D-amphetamine).

* $P < 0.001$ differs from rats receiving only the first injection.

The lowest doses of amphetamine, apomorphine, or clonidine that produced significant decreases in body temperature (that is, 1.4°–1.7° C) were 5.0 mg/kg, 1.5 mg/kg, and 0.5 mg/kg, respectively. The administration of 4.0 mg/kg of pimozide partially suppressed the hypothermia caused by amphetamine (7.5 mg/kg); 50 mg/kg of pimozide totally blocked the hypothermia that followed apomorphine (1.5 mg/kg) or clonidine (0.5 mg/kg). Hypothermia was not observed following D-amphetamine in animals pretreated 14 and 13 days earlier with intracisternal 6-hydroxydopamine (two doses, each 200 μ g) (Table 2), an agent that selectively damages central catecholamine neurones^{5,6}. These observations suggested that the hypothermic response to D-amphetamine is mediated by the release of dopamine from brain neurones. In confirmation of this hypothesis, D-amphetamine still caused significant hypothermia among animals pretreated with drugs that block noradrenergic receptors (phenoxybenzamine, 20 mg/kg, or propranolol, 12 mg/kg) or serotonergic receptors (methysergide, 1 g/kg), as well as by drugs that decrease brain serotonin content (p-chloramphetamine, 5 mg/kg)⁷, damage brain

serotonergic neurones (5,6-dihydroxytryptamine, 75 µg into the cerebrospinal fluid)⁸, or destroy peripheral sympathetic neurones (6-hydroxydopamine, given intraperitoneally, in two doses of 150 mg/kg) (Table 2). Most of those drugs produced hypothermia when given alone and tended to enhance the hypothermia that followed D-amphetamine (Table 2).

The L-isomer of amphetamine also caused hypothermia among animals kept in the cold; its potency was about one-third that of the D-isomer. Colonic temperature fell by 1.9° C among rats receiving 7.5 mg/kg of D-amphetamine or 20 mg/kg of L-amphetamine, and by 2.6° C among animals receiving 15 mg/kg of D-amphetamine or 50 mg/kg of L-amphetamine. The relative potencies of D and L-amphetamine in producing other behavioural or physiological effects thought to be mediated by dopamine are about 1:2; however, L-amphetamine is only one-tenth as potent as D-amphetamine in causing responses thought to be mediated by brain noradrenaline⁹.

Preliminary observations suggest that lesions destroying the limbic dopaminergic neurones projecting from the ventral tegmental nuclei to the region of the olfactory tubercles also block D-amphetamine hypothermia. These lesions do not alter the hyperthermia that follows D-amphetamine administration to rats kept at 20° C or 37° C.

Central dopaminergic neurones mediate both the stereotypic behaviour caused by D-amphetamine^{10,11} and the turning behaviour of rats receiving D-amphetamine after unilateral lesions of the nigro-neostriatal tract¹². Our results suggest that central dopaminergic neurones are also involved in thermoregulation and mediate the hypothermia of D-amphetamine-treated rats kept in the cold; this latter phenomenon may provide a relatively simple tool for screening drugs that interact selectively with dopaminergic brain neurones and their receptors.

These studies were supported in part by a research grant from the US Public Health Service and by a grant from the Alfred P. Sloan Foundation to MIT. We thank Dr Lennart Heimer for making brain lesions, and Dr Ray W. Fuller, Eli Lilly Research Laboratories, for β,β-difluoroamphetamine. Other drugs were provided by: the Smith, Kline and French Co. (D-amphetamine, phenoxybenzamine), Lakeside Laboratories (ditran), McNeil Laboratories (haloperidol and pimozide), Ayerst Laboratories (propranolol), Sandoz Pharmaceuticals (methysergide), and Böhringer Co. (clonidine).

SHLOMO YEHUDA
RICHARD J. WURTMAN

Laboratory of Neuroendocrine Regulation,
Department of Nutrition and Food Science,
and Department of Psychology,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Received September 5; revised October 5, 1972.

- ¹ Yehuda, S., and Wurtman, R. J., *Life Sci.*, (I) **11**, 851 (1972).
- ² Fuxe, K., and Ungerstedt, U., in *Amphetamine and Related Compounds* (edit. by Costa, E., and Garattini, S.), 447 (Raven Press, New York, 1970).
- ³ Anden, N.-E., in *Amphetamine and Related Compounds* (edit. by Costa, E., and Garattini, S.), 257 (Raven Press, New York, 1970).
- ⁴ Anden, N.-E., Corrodi, H., and Fuxe, K., *Europ. J. Pharmacol.*, **17**, 97 (1972).
- ⁵ Thoenen, H., and Tranzer, J. P., *Arch. Pharmacol. Exp. Pathol.*, **261**, 271 (1968).
- ⁶ Lytle, L. D., Shoemaker, W., Cottman, K. T., and Wurtman, R. J., *J. Pharmacol. Exp. Ther.* (in the press).
- ⁷ Biel, J. H., in *Amphetamine and Related Compounds* (edit. by Costa, E., and Garattini, S.), 3 (Raven Press, New York, 1970).
- ⁸ Baumgarten, H., Bjorklund, A., Hostein, A. F., and Nobin, A., *Z. Zellforsch. Mikroskop. Anat.*, **129**, 256 (1972).
- ⁹ Snyder, S. H., Tylor, K. M., Coyle, J. T., and Meyerhoff, L. J., *Amer. J. Psychiat.*, **127**, 117 (1970).
- ¹⁰ Raunrap, A., and Scheel-Krüger, J., *J. Pharm. Pharmacol.*, **18**, 752 (1966).
- ¹¹ Simpson, B. A., and Iversen, S. D., *Nature New Biology*, **230**, 30 (1971).
- ¹² Ungerstedt, U., *Acta Physiol. Scand.*, Suppl. No. 367 (1971).

GABA and Glutamate in Different EEG Stages of the Penicillin Focus

THE penicillin focus has been widely used as an experimental model in the study of epilepsy on which most of our knowledge about the electrophysiological cellular mechanisms of this disease is based. According to current views epileptic phenomena are related to an impairment of excitation-inhibition relationships in the brain. Transition from the inter-ictal stage to seizures results from an abolishment or weakening of inhibitory effects¹. Gamma-aminobutyric acid (GABA) was strongly implied in cortical inhibitory mechanisms and is thought to act as an inhibitory neurotransmitter^{2,3}; glutamate has excitatory properties and is a possible excitatory neurotransmitter². Estimations of GABA and glutamate contents in the brain have been made in animals in which convulsions were induced by convulsive hydrazides, pentamethylenetetrazole, picrotoxin, methionine sulfoximine and high oxygen pressure⁴⁻¹⁰. Berl and Waelsch¹¹ compared levels of GABA and glutamate in a cortical spiking focus, produced by freezing, with levels in the surrounding tissue. We demonstrate here levels of GABA and glutamate in penicillin produced cortical foci, in the inter-ictal and ictal stages respectively, as compared with levels in control animals.

Young cats were used in these experiments. Large craniotomies were performed in *cerveau isolé* animals prepared under ether anaesthesia. After conclusion of surgery, ether administration was interrupted and pain areas were repeatedly infiltrated with 'Novocaine'. EEG recordings from the suprasylvian gyrus were started at least 1.5 h after ether interruption. In control animals cortical samples were taken from the area of EEG recording. In five animals, control samples were taken from one hemisphere, penicillin was applied one hour later on the symmetrical area of the other hemisphere, and after establishment of the focus a second sample was taken. No difference was found in analysis between control samples taken by the two methods, or between "epileptic" samples taken from animals with the opposite hemisphere lesioned by the control sampling. The epileptic foci were produced by loosely applying cotton pledgets (5-6 mm) soaked in K penicillin G solution on the cortex. EEG recordings were taken before application of penicillin, and continuously afterwards until the desired stage, inter-ictal or ictal, of epileptiform activity. Various concentrations of penicillin were used to obtain the two EEG stages. At the required time the electrodes were raised, a large piece of dry ice was applied on the cortex, and a frozen sample of cortex was cut out with a specially shaped sharp knife. Care was taken to cut out the piece of cortex from within the area previously covered with the penicillin soaked cotton pledget. The samples were approximately 3 mm thick and contained mainly grey matter. Only a few seconds elapsed from the removal of electrodes to application of the dry ice and freezing of the cortex, so we are certain that the samples were taken during the course of the electrographic seizures. The frozen tissue pieces were weighed and immersed in a measured volume of ice-cold 80% ethanol. The total GABA and glutamate contents in the samples were determined after separation by paper chromatography and staining with ninhydrin¹².

Table 1 summarizes our results. Control values of GABA and glutamate are within the range of reported data^{13,14}. In the inter-ictal stage GABA concentration decreased significantly ($P < 0.01$), suggesting that inhibitory mechanisms are weakened in this stage and that GABA is involved in these inhibitory mechanisms. Our result is different from that of Berl and Waelsch¹¹, who found no change in GABA level after approximately the same time of spiking in the freezing focus (2 h) as in our experiments. This difference may result from a more intense epileptic process in the penicillin focus.

In the ictal stage we found GABA levels decreased, compared with the controls, but similar to those found in the inter-ictal stage. Intracellular studies have shown that in the

Table 1 GABA and Glutamate Concentrations in Penicillin Focus

Stage of penicillin focus	GABA ($\mu\text{mol g}^{-1}$)	Glutamate ($\mu\text{mol g}^{-1}$)
Control	3.3 ± 0.2 (5)	12.9 ± 1.2 (4)
Inter-ictal stage	2.3 ± 0.1 (5)†	8.8 ± 0.9 (4)*
Ictal stage	2.2 ± 0.1 (11)‡	5.8 ± 0.4 (11)‡

* Difference from control cats significant $P < 0.05$ by t -test.

† $P < 0.01$.

‡ $P < 0.001$.

Results are expressed as mean $\pm$ s.e.; numbers in parentheses represent number of samples. Each sample is composed of tissue taken from one animal.

penicillin focus during seizures neurones are depolarized and this sustained depolarization is probably the result of a fall in inhibition¹. On this basis we expected a further significant decrease of GABA level during seizures and were surprised to find no difference between the inter-ictal and the ictal stages. Several authors have reported decreases of GABA levels during epileptiform states induced by various means^{4-7,9,10}. No change in these levels and observations that an increase in brain GABA is not always correlated with an elevation in convulsive threshold have also been reported^{7,15-18}. Elliott demonstrated a lack of correlation between the total GABA level in the brain and the seizure activity. As GABA is found in the brain in three forms, occluded, bound and free, estimation of the total GABA level may not be indicative of changes in the synaptically active form¹⁹. Our results from the seizure stage are in agreement with this, but we are unable to explain at present why the GABA level did change in the inter-ictal stage.

Glutamate level decreased significantly both in the inter-ictal and the ictal stages ($P < 0.05$ and $P < 0.001$, respectively), the decrease being much more pronounced in the latter stage. Decrease in glutamate during convulsions induced by other means, including the freezing spiking focus, was reported in animals^{4,9,11}. Similarly, a decrease in glutamate was found in the brain samples taken from epileptic patients^{4,20}. The decrease in glutamate can be attributed, at least in part, to the decrease in glucose under the effect of penicillin. Brain glucose decreases during seizures^{4,7}, and energy metabolism is impaired during seizure activity in the penicillin focus²¹. Alternatively, glutamate may participate in the binding of ammonia known to be formed during seizures^{22,23}. As GABA is formed from glutamate²⁴, the decrease in the latter would also explain the decrease in GABA in the inter-ictal stage. We cannot explain, however, the lack of change in GABA in the ictal stage despite a further decrease in glutamate.

ZEHAVA GOTTESFELD
ZEEV ELAZAR *

Isotope Department,
The Weizmann Institute of Science,
Rehovot;
Department of Physiology and Pharmacology,
Tel-Aviv University Medical School,
Tel-Aviv

Received August 30, 1972.

* Present address: Department of Physiology and Pharmacology, Tel-Aviv University Medical School, Beilinson Hospital, Petah Tikva.

¹ Ayala, G. F., Matsumoto, H., and Gummit, R. J., *J. Neurophysiol.*, **33**, 73 (1970).

² Krnjevic, K., *Nature*, **228**, 119 (1970).

³ Bloom, F. E. (edit.), *Neurosci. Res. Bull.*, **10**, 2 (1972).

⁴ Tower, D. B., *Neurochemistry of Epilepsy* (Thomas, Springfield, 1960).

⁵ Killam, K. F., *J. Pharmac. Exp. Ther.*, **119**, 263 (1957).

⁶ Roa, P. D., Tews, J. K., and Stone, W. E., *Biochem. Pharmacol.*, **13**, 477 (1964).

⁷ Nahorsky, S. R., Roberts, D. J., and Stewart, G. G., *J. Neurochem.*, **17**, 621 (1970).

⁸ Saito, Sh., and Tokunaga, Y., *J. Pharmac. Exp. Ther.*, **157**, 546 (1967).

⁹ Tews, J. K., and Stone, W. E., *Prog. Brain Res.*, **16**, 135 (1965).

¹⁰ Wood, J. D., and Watson, W. J., *Can. J. Biochem. Physiol.*, **41**, 1907 (1963).

¹¹ Berl, S., and Waelsch, H., *J. Neurochem.*, **3**, 161 (1958).

¹² Strasberg, P., and Elliott, K. A. C., *Can. J. Biochem.*, **45**, 1795 (1967).

¹³ Popov, N., Pohle, W., Rösler, V., and Matthies, H., *Acta Biol. Med. Ger.*, **18**, 695 (1967).

¹⁴ McIlwain, H., and Bachelard, H. S., *Biochemistry of the Central Nervous System* (Churchill Livingstone, Edinburgh, 1971).

¹⁵ Leonard, B. E., and Palfreyman, M. G., *Biochem. Pharmacol.*, **21**, 1206 (1972).

¹⁶ Baxter, C. F., and Roberts, E., *Proc. Soc. Exp. Biol. Med.*, **104**, 426 (1960).

¹⁷ Maynert, E. W., and Kaji, H. K., *J. Pharmac. Exp. Ther.*, **137**, 114 (1962).

¹⁸ Kuriyama, K., Roberts, E., and Rubinstein, M. K., *Biochem. Pharmacol.*, **15**, 221 (1966).

¹⁹ Elliott, K. A. C., *Jap. J. Brain Physiol.*, **84**, 4 (1967).

²⁰ Van Gelder, N. M., Sherwin, A. L., and Rasmussen, T., *Brain Research*, **40**, 385 (1972).

²¹ Swanson, Ph. D., *Arch. Neurol.*, **26**, 169 (1972).

²² Richter, D., and Dawson, R. M. C., *J. Biol. Chem.*, **176**, 1199 (1948).

²³ Stone, W. E., in Jasper, H. H., Ward, A. A., and Pope, A. (eds.), *Basic Mechanisms of Epilepsies* (Little, Brown, Boston, 1967).

²⁴ Roberts, E., and Frankel, S., *J. Biol. Chem.*, **190**, 505 (1951).

Processes of Synthesis in Visual Perception

ANY spatial distribution of luminance can be considered as the sum of its sinusoidal Fourier components, which are defined when their amplitudes and phases are given. Recently Fourier theory has been applied in describing the transmission of spatial information in the visual system. Indeed neurones selectively sensitive to a narrow band of spatial frequencies have been found in the visual cortex of the cat and monkey^{1,2}. With psychophysical methods, using an adaptation effect, spatial frequency channels have been observed also in the visual system of man^{3,4}.

The most serious criticism of the hypothesis that the visual system works as a Fourier analyser is that there is no indication that the various components can be resynthesized in the brain for the reconstruction of the visual pattern. The mere existence of spatial frequency channels does not mean that the visual system functions according to a Fourier scheme of analysis and synthesis.

We think that this attractive hypothesis would be strengthened if we could show the existence of nervous operators able to perform the synthesis of the retinal image from its sinusoidal components.

When two sinusoidal spatial distributions of light are physically superimposed, one perceives a periodical pattern the luminance profile of which is the sum of the two components and therefore depends on their frequency, relative phase and contrast. In this case one cannot know whether the amplitudes and phases of the two stimulus components are separately transmitted and later synthesized or whether the resultant pattern is perceived as a *Gestalt*, without separate processing of the two components. Suppose now that the two sinusoidal components are projected separately on the two retinæ with their phases adjusted with respect to the two foveae in the same way as they were physically adjusted in the monoptic case. If there is some mechanism in the brain the function of which is to synthesize the harmonics of the retinal images under normal viewing conditions, this mechanism should also work when the harmonics are transmitted separately by the two eyes: the pattern perceived dichoptically should look the same as in monoptic viewing. This would be evidence in favour of a process of analysis and synthesis of the retinal stimulus in terms of its sinusoidal components.

Here we shall show that if two harmonic components of a given periodical pattern are presented separately to the two eyes, one perceives a resultant pattern as predicted by Fourier synthesis.

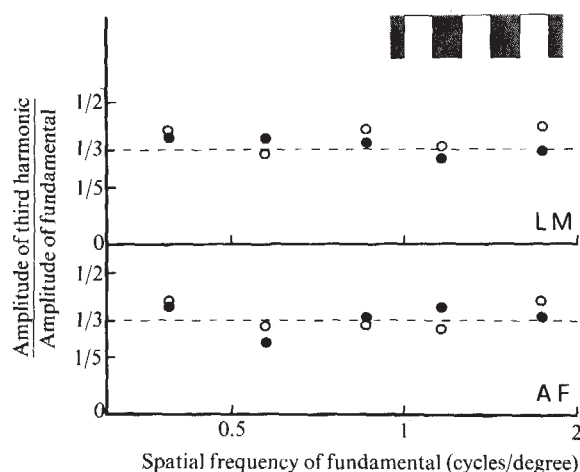


Fig. 1 Perceptual synthesis of the first and third harmonic of a square-wave spatial distribution. Ordinates: contrast of the third harmonic giving the best approximation to a square-wave pattern, divided by the contrast of the fundamental. Both contrasts are expressed in threshold units. Data for two subjects. Standard errors range between 0.02 and 0.08. ●, Dichoptic; ○, monoptic.

It is known that if spatially dissimilar fields are presented to each eye we get either fusion resulting in a depth effect, or binocular rivalry⁵. But here the gratings presented to the two eyes are markedly different, and yet we perceive a fused compound pattern. For the binocular fusion of two homogeneous fields it is already known that the two monocular brightnesses add linearly⁵. But here we shall show linear summation of two patterns with different luminance profiles.

The stimuli were two one-dimensional luminance distributions (vertical sine-wave gratings) electronically generated on the faces of two identical oscilloscopes. The contrast, spatial frequency and phase of the patterns could be varied by means of potentiometers, without changing their average luminance. The average luminance of the screens was 2 cd m⁻². Two fixation points were centred on the two screens, which were viewed from 1 m distance. Viewing was either monoptic, the two screens being optically superimposed by means of a beam splitter, or dichoptic, the two screens being presented separately to the two eyes, one directly and the other by reflexion on a plane mirror. In the dichoptic condition the relative phase of the two sine-waves was determined by their position with respect to the fixation point.

First we tried the binocular synthesis of the first two harmonics of a square-wave distribution. The first two terms of the Fourier series for a square-wave are the fundamental and the third harmonic with an amplitude ratio of 1/3 and with the positive peaks of the fundamental coinciding with the negative peaks of the third harmonic. When the two harmonics are presented dichoptically with their amplitudes and phases suitably adjusted, the observer perceives a pattern which approximates very closely a square-wave distribution, just as if the two components were superimposed physically and viewed monoptically. This was done for low spatial frequencies so that the third harmonic falls in a range of relatively high contrast sensitivity, and with a contrast of the fundamental relatively low, so that the relative contributions of the higher harmonics to the perception of a square-wave were almost negligible. Another advantage of using low contrast is facilitation of binocular fusion: indeed with patterns of low contrast most subjects do not experience prolonged suppressions of one monocular image.

Several subjects unaware of the scope of the experiment were presented with the composed pattern and all of them reported that the brightness distribution perceived dichoptically did not appreciably differ from that perceived monoptically.

For two subjects a quantitative experiment was performed. The contrast thresholds were determined for the fundamental

and for the third harmonic. The fundamental was then set at a contrast five times its threshold and the subject adjusted the contrast of the third harmonic at the value that produced the best approximation to a square-wave pattern. This was done both monoptically and dichoptically.

The results are presented in Fig. 1 for various spatial frequencies of the fundamental. On the ordinates we have reported the ratio of the contrast of the third harmonic to the contrast of the fundamental, both expressed in terms of threshold units. The ratio is as close to 1/3 for the dichoptic as for the monoptic condition. The perceptual synthesis therefore is in agreement with Fourier theory.

We then attempted the perceptual synthesis of another pattern: a rectangular-wave with a duty cycle different from 0.5 (Fig. 2). The Fourier expansion of this pattern contains a second harmonic the amplitude of which depends on the duty cycle while the phase shifts by 180° according to whether the duty cycle is greater or smaller than 0.5. We used duty cycles of 1/3 and 1/4, for which the amplitude of the second harmonic is 0.5 and 0.7 the amplitude of the fundamental, respectively. This distribution was found to be particularly suitable for the present experiment because the width of the light and dark bars in the dichoptic pattern undergoes evident changes when the amplitude of the second harmonic is varied and the widths of the light and dark bars interchange when the phase of the second harmonic is reversed. Fifteen naive subjects readily perceived changes in the dichoptic pattern when the duty cycle was varied or the phase of the second harmonic was reversed.

To investigate the properties of the process of synthesis a

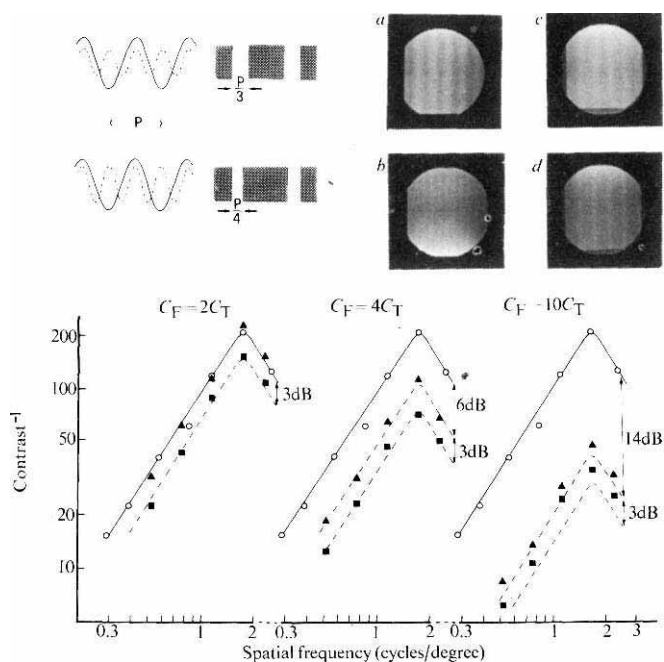


Fig. 2 Perceptual synthesis of the first and second harmonic of a rectangular-wave spatial distribution. Top left: first and second harmonics of rectangular waves with duty cycle of 1/3 and 1/4. Top right: sinusoidal gratings of spatial frequency f (a) and $2f$ (b). Gratings c and d result from the physical superposition of a and b with amplitude ratios of 1/3 and 1/4 and with the phases indicated on the left. Bottom: The ordinates are reciprocals of grating contrast. The circles represent reciprocals of contrast thresholds C_T . The triangles and the squares represent the reciprocal of the contrast of the second harmonic (in threshold units) required for the width of the bright bars to appear 1/3 and 1/4 of the fundamental period, respectively. The three sets of results were obtained for three different contrasts C_F of the fundamental. S.e. of triangles and squares range between 0.03 and 0.04 log units. ○, Contrast sensitivity (reciprocal of the contrast threshold) in the range of frequency investigated. The points were fitted by hand (—). ▲ and ■, Reciprocal of the contrast of the second harmonic (in threshold units) required for the width of the bright bars to appear 1/3 and 1/4 of the fundamental period, respectively.

more quantitative dichoptic experiment was performed in three subjects. The phase of the second harmonic was such that its positive peaks coincided with the negative peaks of the fundamental. After determining the contrast threshold for the fundamental and the second harmonic, the contrast of the fundamental (C_F) was set by the operator at a value 2, 4 or 10 times its threshold C_T . Then the subject was required to adjust the contrast of the second harmonic so that the width of the bright bars appeared to be approximately 1/3 or 1/4 the period of the pattern. Measurements were made for five different frequencies of the fundamental.

The results are reported in the lower part of Fig. 2 for one subject. The results for which the contrast of the fundamental was twice the threshold are reported in the left part of the figure. In this case, according to Fourier theory, the expected amplitude of the second harmonic should be at threshold (0.5 the fundamental amplitude) for a duty cycle 1/3 and 3 dB above threshold (0.7 the fundamental amplitude) for a duty cycle 1/4.

This expectation is fulfilled by both sets of experimental results: indeed, at each spatial frequency investigated the triangle falls on the contrast sensitivity curve and the square falls 3 dB (0.7/0.5) below. The dotted line is the contrast sensitivity curve displaced 3 dB downwards. This is true also for the analogous set of points in the central part of the figure, where the contrast of the fundamental is four times its threshold. For a contrast of the fundamental ten times its threshold (right part of the figure) the fitting is not satisfactory. Similar results were obtained from the other two subjects.

We have shown that the information about the amplitude and phase of two sinusoidal stimuli presented separately to the two eyes can be synthesized by the visual system. The quantitative results indicate that this process of synthesis can be described with good approximation in terms of Fourier theory, at least for relatively low contrast. In the introduction we mentioned previous evidence suggesting that a Fourier analysis of the retinal stimuli is performed by the visual system. The present findings have shown the existence of nervous operators able to rebuild the image from its sinusoidal components. The localization of these operators is at or beyond the level of binocular fusion.

L. MAFFEI
A. FIORENTINI

Laboratorio di Neurofisiologia del CNR,
Pisa

Received August 2, 1972.

¹ Campbell, F. W., Cooper, G. F., Robson, J. G., and Sachs, M. B., *J. Physiol.*, **204**, 120 (1969).

² Campbell, F. W., Cooper, G. F., and Enroth-Cugell, Christina, *J. Physiol.*, **203**, 223 (1969).

³ Blakemore, C., and Campbell, F. W., *J. Physiol.*, **203**, 237 (1969).

⁴ Campbell, F. W., and Maffei, L., *J. Physiol.*, **207**, 635 (1970).

⁵ Levelt, W. J. M., *On Binocular Rivalry* (Mouton, The Hague, 1968).

Unilateral Inhibition of Audiogenic Seizures and Preyer Reflexes

EXPOSING a mouse to a loud acoustic stimulus for 30 s during a critical period of neural development will alter the animal so that a subsequent exposure days or weeks later results in convulsions which often terminate in death¹. The effects of this first (priming) exposure require several days before they are overtly manifest. Fuller and Collins² have shown that re-exposure to loud sounds every 6–12 h after priming delays the onset of convulsibility for as long as the procedure is continued, and that this effect can be lateralized within the nervous system³. This paper extends their findings by the use of a modified form of their technique which inhibited the fully developed effects of priming, and demonstrates that acoustic inhibition increases the threshold to the acoustic startle

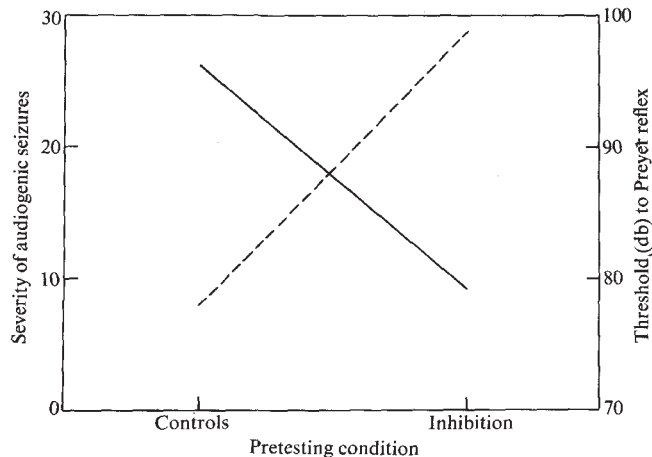


Fig. 1 Severity of audiogenic seizures (—) and threshold to Preyer acoustic startle response (---) in acoustically primed C57BL/6J mice. Bilateral acoustic inhibition 4 h before testing a single ear reduces severity of seizures and elevates the threshold of the acoustic startle response.

response by 21 dB, and that this effect can be lateralized to a single side of the auditory system.

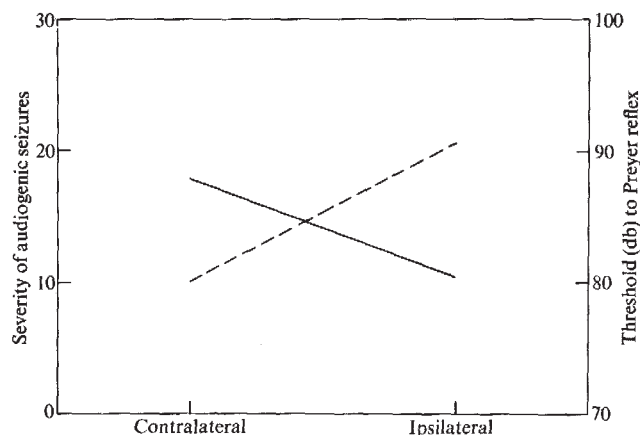
Forty mice were acoustically primed at 16 days of age. Five days later, they were anaesthetized with ether and nineteen of the subjects were re-exposed to the priming bell (114 dB re. 2×10^{-5} N/m²) for 30 s. The anaesthesia protected the mice from convulsions. Four hours later, all mice were examined for thresholds to the Preyer acoustic startle reflex with glycerine plugging either their left or right ear. It was determined electrophysiologically that this attenuated the sound by at least 20 dB. The auditory stimuli were 1 s bursts of narrow band noise (10 kHz–20 kHz) with a rise and decay of 10 ms. Immediately after threshold determination, they were tested for audiogenic seizures with glycerine still plugging either their right or left ear.

As demonstrated by the audiogenic seizure severity scores⁴ (Fig. 1), this procedure effectively inhibited audiogenic seizures (from 26.19 to 9.21; $t=3.37$; $df=38$; $P<0.0005$). Latency to wild running was also increased (from 3.0 to 7.4 s; $t=3.58$; $df=24$; $P<0.0005$), as was the threshold of the Preyer reflex (from 77.93 dB to 98.84 dB; $t=12.9$; $df=39$; $P<0.00001$), suggesting that the protection afforded by acoustic inhibition was due to a decrease of auditory sensitivity.

The laterality of this effect was examined in a second group of forty-five primed C57BL/6J mice. Unilateral inhibition was achieved by plugging one ear with glycerine, anaesthetizing the mice with ether, and exposing the 21-day-old subjects to the bell for 30 s. Four hours later, either the same (ipsilateral) or the opposite (contralateral) ear was plugged and the subjects were tested as in the first experiment (Fig. 2). An ipsilateral effect was seen for seizure severity (17.9 against 10.4; $t=2.05$; $df=43$; $P<0.01$) and for latencies (10.7 against 3.26 s; $t=3.8$; $df=23$; $P<0.0005$). Again, decreased seizure severity and increased latencies were associated with an increase of the Preyer threshold (from 80.07 to 90.63 dB; $t=2.77$; $P<0.005$).

To determine whether this inhibitory effect is unique to primed mice or whether nonprimed subjects also show a Preyer threshold elevation, a third experiment was conducted. Thirty-five 21-day-old nonprimed C57BL/6J mice, seventeen of which had been bilaterally inhibited 4 h earlier, were bilaterally tested for Preyer reflex threshold. Inhibition also occurred in these nonprimed mice, raising the Preyer threshold from 80.9 dB to 92.8 dB ($t=5.67$; $df=33$; $P<0.001$). This 10.9 dB inhibition for nonprimed mice was considerably less than the 20.8 dB inhibition observed in the primed subjects of experiment 1.

The laterality of the inhibitory effect on both seizures and the Preyer threshold and the fact that inhibition of a lesser degree can also occur in nonprimed mice suggest a mechanism



Relationship of ears acoustically inhibited and tested

Fig. 2 Severity of audiogenic seizures (—) and threshold to Preyer reflex (---) in acoustically primed C57BL/6J mice. Testing was performed in a single ear 4 h after the same (ipsilateral) or the opposite (contralateral) ear was acoustically inhibited. Both effects show a unilateral response.

not specific to priming. Priming a 16-day-old mouse will produce a 20 dB increase of the absolute auditory threshold and a larger amplitude evoked auditory potential in response to 100 dB sounds, but these effects have only been examined in 21-day-old subjects⁵. Acoustic inhibition may produce a nonspecific change in one or both of these two parameters. Since the behavioural and physiological effects of priming closely resemble those seen in humans with recruitment deafness⁵, further studies of acoustic inhibition may reveal information concerning this condition.

This work was supported by a grant from the US National Science Foundation.

KENNETH R. HENRY
JAMES F. WILLOTT

Department of Psychology,
University of California,
Davis, California 95616

Received May 15; revised August 10, 1972.

¹ Henry, K. R., *Science*, **158**, 938 (1967).

² Fuller, J. L., and Collins, R. L., *Science*, **162**, 1295 (1968).

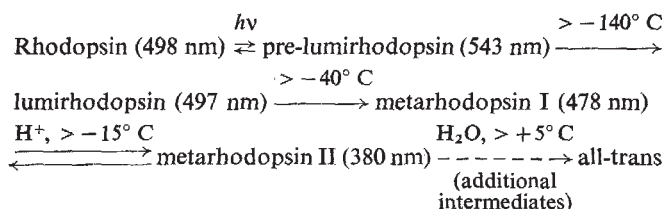
³ Collins, R. L., *Science*, **167**, 1010 (1970).

⁴ Henry, K. R., and Bowman, R. E., *J. Comp. Physiol. Psychol.*, **70**, 235 (1970).

⁵ Henry, K. R., and Saleh, M., *J. Comp. Physiol. Psychol.* (in the press).

Nanosecond Laser Photolysis of Rhodopsin in Solution

LIGHT absorption by the rhodopsin molecule initiates the visual process¹⁻³. The absorption of light starts a series of consecutive reactions in which the system passes through a number of intermediates leading to the final products, opsin and all-trans retinal. Stages in the bleaching of rhodopsin are well known and believed to be associated with the electrical events in the retina



retinal (387 nm) + opsin

The various intermediates were first identified by the spectral changes induced by exposing rhodopsin to steady-state illum-

ination. The observations were carried out at low temperatures at which each of the intermediates was fairly stable. Application of fast flash photolysis techniques makes it possible to study the intermediates at higher temperatures³. The question arises whether the first (apparently) intermediate, pre-lumirhodopsin (543 nm) also initiates the bleaching process at physiological temperatures. Grellmann *et al.*^{4,5} using flash photolysis have pre-lumirhodopsin and its conversion to lumirhodopsin at temperatures as high as -50°C . Cone⁶ reported the detection of pre-lumirhodopsin (1 μs decay) in an excised albino rat retina at 2°C . This observation has not apparently been confirmed or extended to rhodopsin in solution. In our work a 3371 Å N_2 laser photolysis apparatus⁷, with a time resolution of $\sim 10 \text{ ns}$, was used to study the initial stages of rhodopsin photochemistry at physiological temperatures.

Dark adapted cattle retinas were supplied in dry ice (G. A. Hormel, Minnesota). Rhodopsin samples were prepared from 100 retinas using the method of Matthews *et al.*⁸. Preparation of outer rod segments was extracted with petroleum ether, and rhodopsin was rendered soluble by homogenization with 3–4 ml. of 2% 'Triton X100'. After centrifugation the ratio between the absorbance at 400 nm and that at 500 nm¹⁰ was 0.17 confirming the purity of the final solution.

Pulsed photolysis experiments at room temperature were carried out on rhodopsin solutions with an absorbance of ~ 1 (1 cm cell) at the 337.1 nm laser line (~ 4 at 498 nm). The high optical densities of the solutions in the region of the α (498 nm) rhodopsin band were due to the 337.1 nm line being within the low intensity β band of the molecule. This limited our observational range to above $\sim 575 \text{ nm}$. Excitation led to a transient change in absorbance, exhibiting a $\sim 50 \text{ ns}$ decay and a spectrum peaking around 580 nm (Fig. 1). The wavelength range limitation, as well as the relatively low signal to noise ratio, prevented an accurate determination of the location of maximum change. The estimated peak value around 580 nm, and the 650 nm threshold, are in agreement with the initial difference spectra observed in the photobleaching of rhodopsin at -75°C (flash excitation)^{4,5} and -195° (steady illumination). Our absorbance-change spectrum is redshifted by approximately 15 nm relative to those observed at low temperatures by Grellmann *et al.*⁴. This may be due to a temperature dependence of the pre-lumirhodopsin absorption, or experimental artefact due to the steep rise in the rhodopsin absorbance below 590 nm. The long wavelength contribution to the light transmitted at a determinate setting of the monochromator (slits: $\sim 0.8 \text{ nm}$) may exceed the short wavelength side. This may be finally reflected as a small redshift in the

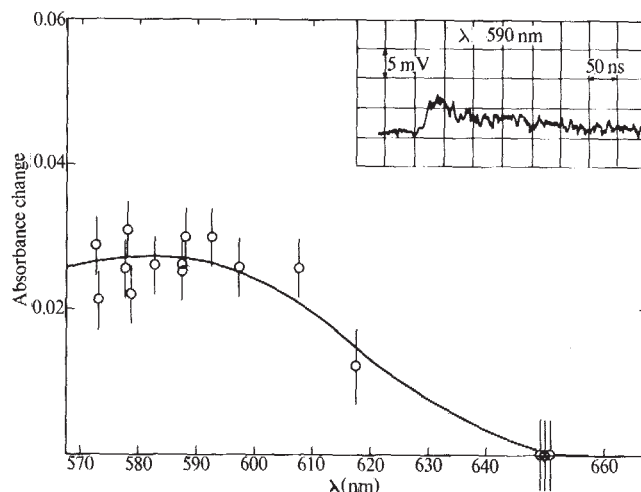


Fig. 1 Oscillogram (inset) showing the decay attributed to pre-lumirhodopsin in the N_2 laser photolysis of a rhodopsin solution at room temperature. The light to dark deflexion was 120 mV. The spectrum represents the absorbance change recorded 15 ns after the rise of the laser pulse.

location of the absorption peak. The values represent the absorbance difference between rhodopsin and pre-lumirhodopsin. The observed decay time of ~ 50 ns is within the time range predicted by extrapolation of the low temperature data⁵, although it is not known whether the room temperature decay is also multistaged.

The bleaching of rhodopsin is initiated by light absorption within the γ (protein) band¹¹ as well as within the α and β bands¹². After lens removal in cataract operations, human vision extends down to around 312 nm. It is therefore very likely that the present conclusions, following excitation at 337.1 nm, can be extended to illumination within the visible α band. Pre-lumirhodopsin appears to be a primary intermediate in the rhodopsin photochemistry at temperatures similar to physiological conditions. The upper time limit now set for its evolution after excitation is equal to the resolution of the laser photolysis system, that is, around 10 ns.

We thank Dr Y. Elkana and Professors G. Forti, Y. Ohad and H. Berman for discussions.

T. ROSENFELD
A. ALCHALAL
M. OTTOLENGHI

Department of Physical Chemistry,
The Hebrew University,
Jerusalem

Received August 8, 1972.

- ¹ Hubbard, R., Bownds, D., and Yoshizawa, T., *Cold Spring Harbor Symp. Quant. Biol.*, **30**, 301 (1965).
- ² Wald, G., *Science*, **162**, 230 (1968).
- ³ Abrahamson, E. W., and Ostroy, S. E., *Prog. Biophys. Mol. Biol.*, **17**, 179 (1967).
- ⁴ Grellmann, K. H., Livingston, R., and Pratt, D., *Nature*, **193**, 1258 (1962).
- ⁵ Pratt, D. C., Livingston, R., and Grellmann, K. H., *Photochem. Photobiol.*, **3**, 121 (1964).
- ⁶ Cone, R. A., lecture; see Spector, A., *Arch. Ophthalmol.*, **83**, 506 (1970).
- ⁷ Goldschmidt, C. R., Ottolenghi, M., and Stein, G., *Israel J. Chem.*, **8**, 29 (1970).
- ⁸ Matthews, R. G., Hubbard, R., Brown, P. K., and Wald, G., *J. Gen. Physiol.*, **47**, 215 (1963).
- ⁹ Zorn, M., and Futterman, S., *J. Biol. Chem.*, **246**, 881 (1971).
- ¹⁰ Shields, J. E., Dinovo, E. C., Henriksen, jun., R. A., Kimbel, R. L., and Millar, P. G., *Biophys. Acta*, **147**, 238 (1967).
- ¹¹ Kropf, A., *Biophys. J.*, **5**, 75 (1965).
- ¹² Goodeve, C. F., Lythgoe, R. J., and Schneider, E. E., *Proc. Roy. Soc., B*, **130**, 380 (1942).

Partial Cell Irradiation with a Tunable Organic Dye Laser

SINCE the laser first became available, it has been applied extensively for partial cell irradiation¹⁻⁴. The intensity, coherence and monochromaticity of laser light have made it an ideal radiation source for selective alteration of cell organelles. Most of the laser microbeam studies up to the present have utilized a laser device that emits light of either a single or small number of wavelengths. In a few instances it has been possible to increase the spectral output of a laser by using non-linear optics to generate second or third harmonic wavelengths^{5,6}. Ideally, an investigator would like one microbeam system that can deliver wavelengths throughout the entire visible and ultra-violet spectrum. Such a device would permit alteration of a greater variety of organelles and organisms, and it would facilitate derivation of the precise action spectrum of the particular response being studied. This capability would be invaluable for understanding the basic nature of the biological change at the molecular level.

Here I describe a laser microbeam system that uses an organic dye laser as the radiation source. By changing the dye and/or the concentration of the dye, it is possible to generate high energy at any wavelength in the visible region of the spectrum.

A detailed diagram of the apparatus is presented in Fig. 1.

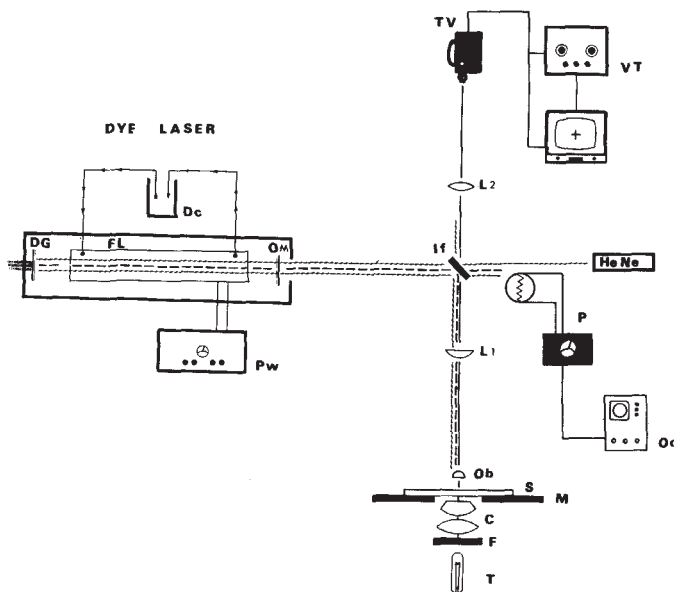


Fig. 1 Dye laser microbeam system. DC, Dye container; DG, diffraction grating; FL, flash lamp; OM, output mirror; Pw, laser power supply; TV, television camera; VT, videotape recorder; L₂, focal length image correction lens; If, dichroic filter; L₁, X1 ocular; Ob, microscope objective (either Zeiss $\times 40$ or $\times 100$ Neofluar); S, specimen chamber; M, microscope stage; C, microscope condenser; F, substage filter; T, tungsten light source; HeNe, helium neon laser alignment beam; P, power meter; Oc, oscilloscope; ---, dye laser beam; . . . , substage illumination containing specimen image; ~~~~, alignment laser beam; dye laser purchased from Synergetics Inc., Princeton, New Jersey.

The five major components of the system are (1) the dye laser, (2) a phase microscope, (3) a recording system (television camera, monitor and videotape), (4) the HeNe laser alignment system, (5) an energy recording device. Only the first two components (dye laser and microscope) are absolutely essential, though the other components allow more efficient data collection and analysis. The dye is circulated from an external container, containing 300–500 ml. of dye solution, through a coaxial xenon flash lamp in the laser cavity. The rear reflector may be either a broad band reflecting mirror or a diffraction grating. A broad band reflector results in a laser emission of several hundred angstroms spread that is characteristic of the particular dye. The use of a diffraction grating permits fine wavelength tunability (± 1 Å). The output of the dye laser is monitored with a calibrated vacuum photodiode. Measurements in the focal plane of the $\times 100$ objective indicated that 1–10% of the total laser output was transmitted through the optical system and into the focused spot.

Four different organic dyes dissolved in absolute methanol were used: coumarin (7-diethylamino-4-methyl coumarin), fluorescein (2-7-dichlorofluorescein), rhodamine 6G and acridine red. It is evident (Fig. 2) that a wide variety of wavelengths from the blue to the red regions of the spectrum can be produced. The only gap is between 480 and 530 nm, and a lasing dye will be available soon for that region of the spectrum. It is also of interest to note that the emission spectrum of fluorescein, rhodamine 6G and acridine red can be shifted to longer wavelengths by increasing the dye concentrations. This is important since inadvertent changes in concentrations (due to inaccurate measuring, or ageing dye solutions and solvent evaporation) may result in a shift in the emission wavelengths of the laser.

Outputs varied from a mean of 60 mJ in the yellow-green for fluorescein to a mean of 150–200 mJ in the orange-red for rhodamine. Pulse duration for all dyes was 200 ns, therefore at times it was possible to obtain a peak power of 1 megawatt for the rhodamine dye. For a 1 μ m focused spot and a 1–10% transmission through the microscope a total power density of 10^5 W μ m⁻² is attainable.

To demonstrate that it is possible to produce biological lesions as small (less than 1 μm diameter) as those previously described with the argon laser, human red blood cells were irradiated with threshold (that amount of energy just capable of providing a visible lesion) laser energies from the coumarin dye. Lesions of 0.5 μm were produced repeatedly.

To test the apparatus on live biological systems, three different organelles were irradiated. The mitotic chromosomes of salamander lung epithelium were irradiated through the $\times 100$ objective. Total energy in the one micron focused spot was 0.05–0.5 mJ. The cultures were established and handled identically to those procedures described for the argon laser⁷. No vital dye was applied to the cells to sensitize them to the laser light. Following irradiation with the dye laser a one micron segment of the chromosome exhibited the typical phase "paling" reaction (Fig. 3a) that has been described and analysed extensively. The paling reaction was much more intense, and appeared more rapidly following dye laser irradiation than that obtained by argon laser irradiation. It was possible to obtain the same degree of paling with the dye laser as observed with the argon laser by attenuating the beam energy by half. It was also possible to run an action spectrum of the paling response by irradiating with comparable energy levels with wavelengths from the other dyes. The paling response was only obtained when the blue wavelengths from the coumarin dye were used, even when considerably higher energies ($\times 10$ –100) of the yellow, orange and red wavelengths were employed. This particular study will be published in detail elsewhere.

A second organelle irradiated was the large mitochondrion (1–4 microns in diameter) of contracting rat myocardial cells *in vitro*. Previously this organelle had been irradiated with the argon laser⁸, second harmonic of the neodymium laser⁹, and the ruby laser (Berns, unpublished). The dye laser wavelengths from both coumarin and rhodamine were used. Comparable alterations in both morphology (Fig. 3b, c) and contractility were produced.

The third organelle irradiated with the dye laser was the large single chloroplast in the cells of the blue-green alga *Coleochaete*. Laser wavelengths of both coumarin (blue) and rhodamine (orange) were used. Because of the efficient absorption by the chloroplast it was possible to destroy an entire cell in the thallus (Fig. 3d) by irradiation with only a medium amount of blue or orange laser light. Using lower laser intensities it was possible to destroy only the chloroplast or a portion of the chloroplast in a single cell. This particular approach is being used to study the development and functional interactions within the thallus (with G. McBride, Department of Botany, University of Michigan).

The versatile microbeam device described here combines all the features of previous laser microbeams. In addition, the commercial availability of non-linear optical crystals for placement directly within the laser cavity now makes available

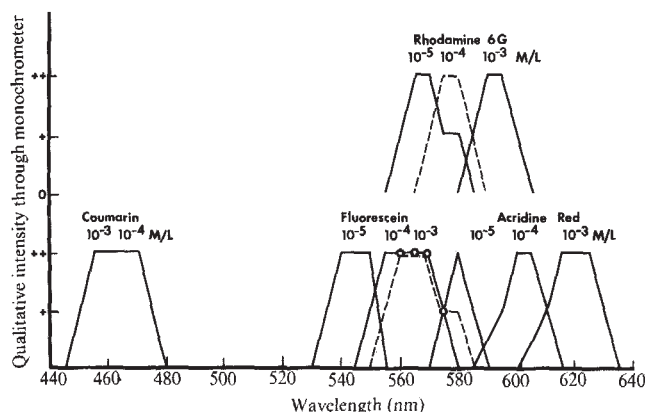


Fig. 2 Spectral output of various laser dyes tested; qualitative energy designations (O, +, ++, +++) obtained by visual evaluation of the beam passing through a monochromator.

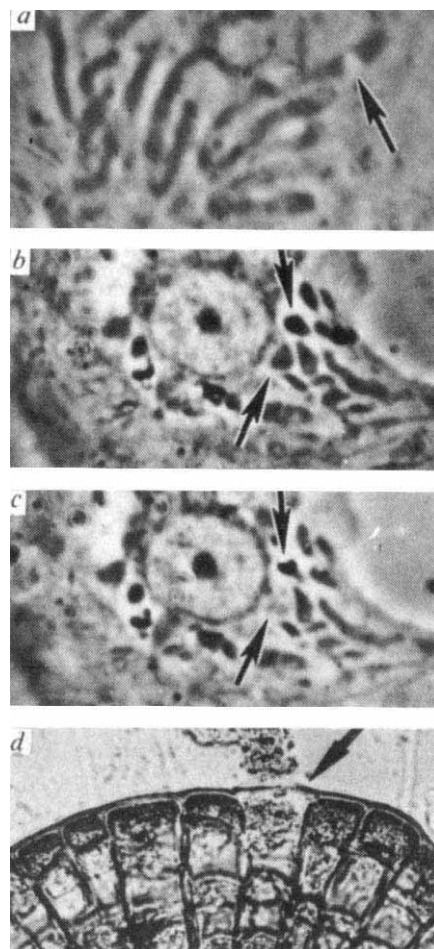


Fig. 3 a, Early metaphase set of salamander (*Taricha*) chromosomes after irradiation with blue laser light from coumarin dye. Note the chromosome "paling". Energy in the focused spot was 0.05–0.5 mJ. ($\times 2,000$.) b, Rat myocardial cell *in vitro* before irradiation; arrows indicate two large mitochondria. c, Same cells several minutes post-irradiation; one mitochondrion appears to have "paled" and the other appears to have been condensed (coagulated?). Cell survival was not impaired and contractions continued unaltered. ($\times 1,500$.) d, Blue-green alga *Coleochaete* after irradiation of large chloroplast with blue laser light; high magnification of damaged cell (arrow); note other cells appear unaltered; apparently cell wall was ruptured and cellular material ejected. ($\times 500$.)

the second harmonic (one half the wavelength) of every available major visible wavelength. Thus, one has complete tunability in the ultraviolet regions as well. The fact that organelles as diverse as chloroplasts, chromosomes and mitochondria, from organisms as different as the rat, salamander and alga, can be effectively probed with a single microbeam device should suggest applications to a large number of biologists.

This work was supported by grants from the US National Science Foundation, Genetic Biology Program, and the Heart and Lung Institute of the US National Institutes of Health.

M. W. BERNs

Department of Zoology,
University of Michigan,
Ann Arbor,
Michigan 48104

Received July 17; revised August 28, 1972.

- 1 Bessis, M., and Ter-Pogossian, M. M., *Ann. NY Acad. Sci.*, **122**, 689 (1965).
- 2 Moreno, G., Lutz, M., and Bessis, M., *Int. Rev. Exp. Pathol.*, **7**, 99 (1969).
- 3 Berns, M. W., Olson, R. S., and Rounds, D. E., *Nature*, **221**, 74 (1969).
- 4 Berns, M. W., and Cheng, W., *Nature*, **233**, 122 (1971).

- ⁵ Moreno, G., Salet, C., and Bessis, M., *CR Acad. Sci. Paris*, **269**, 781 (1969).
⁶ Berns, M. W., Matsui, S., Olson, R. S., and Rounds, D. E., *Science*, **169**, 1215 (1970).
⁷ Berns, M. W., Cheng, W. K., Floyd, A. D., and Ohnuki, Y., *Science*, **171**, 903 (1971).
⁸ Berns, M. W., Gross, D. C. L., Cheng, W., and Woodring, D., *J. Mol. Cell. Cardiol.*, **4**, 71 (1972).
⁹ Salet, C., *Exp. Cell Res.* (in the press).

Photoperiod and Chemical Evidence of the Introduction to India of *Xanthium* from America

THE aggressiveness of Indian populations of *Xanthium* suggested exotic niche capacities of introduced plants. To test this hypothesis, the plants were compared with *Xanthium* from America and from Hong Kong. Effects of photoperiod and temperature conditions were tested and the plants examined chemically. The treatment of *Xanthium*, based largely on burr morphology, is various. The indigenous populations of Europe and Asia are referred to *X. strumarium* L. and closely related species^{1,2} or to the *strumarium* morphological complex³. The indigenous American populations have been referred to numerous species^{1,2} and to seven morphological complexes³. Populations of the southeastern US and the Caribbean islands have been referred to the *chinense* morphological complex³.

Xanthium populations of America within a morphological complex show diverse photoperiodic adaptation⁴⁻⁹. Those of northern US and Canada have critical dark periods of 7.5–8.5 h for reproduction⁴ and those of southern US and Mexico have critical dark periods extending to 10.5–11 h⁵⁻⁷. Populations of intermediate latitudes show intermediacy^{4,6}, and those of desert areas show adaptation to local habitat conditions⁸. Populations with a critical night of 10.5 h in the *chinense* morphological complex are confined in the US to the Mississippi Delta of Louisiana but are of widespread introduction as "Noogoora Burr" in eastern Australia^{7,9}.

Kaul¹⁰ reports four morpho-physiological types of *Xanthium* in India. All four forms may grow together at Varanasi as a result of delayed germination and may give rise to intermediate forms¹⁰. During 1971, *Xanthium* burrs of diverse morphology were collected in various parts of India. Material collected in Hong Kong was representative of the diminutive burrs and leaf morphology of the *strumarium* complex.

To test for flowering response to photoperiod and temperature, diverse burr collections from plants resembling the *strumarium* complex and the *chinense* complex were soaked overnight in a dilute solution of a fungicide (Consan-20) and germinated in sand. After two weeks under continuous light at 30° C day and 24° C night, the seedlings were transplanted individually to 14-ounce polystyrene cups filled with fine sandy loam and given regular nutrient. After seven weeks plants were placed in growth chambers with 9.5 and 10.5 h nights. The nights were lengthened to 11 and 11.5 h by 15 min every week. Two sets of plants were kept at 10.5–11.5 h, one at 30–24° C, the other at 24–15° C. The growth chambers were programmed for 10 h at the higher temperature and a higher light intensity (24,600 lumen/m²) followed by 10 h at the lower temperature and a lower light intensity (825–1,100 lumen/m²) and/or dark period, allowing 4 h for temperature transition. All chambers received 12 h of the higher light intensity from a combination of fluorescent and incandescent lamps. Light period extensions were provided with incandescent lamps only.

The Indian collections with leaf and burr morphology similar to *strumarium* plants flowered under continuous light (Fig. 1a, Table 1) but were influenced by temperature. This flowering response was reported by Kaul¹⁰ for a "summer" form of the Indian plants. Plants two weeks from sowing produced macroscopic floral buds (100%) after another two weeks at 30–24° C but gave a similar response after another five weeks at 24–15° C.

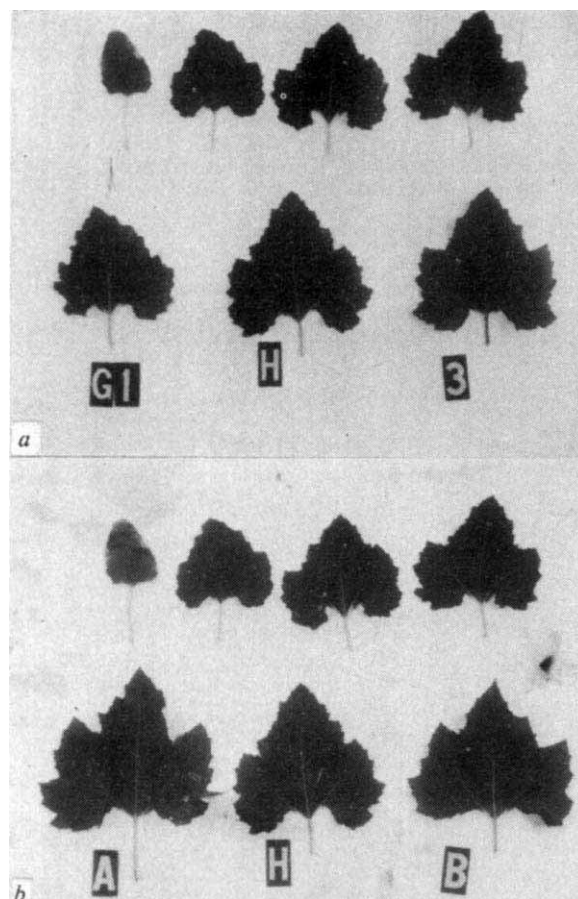


Fig. 1 a, Comparison of *Xanthium* leaves under controlled conditions. Top row, left to right, developmental series, Hong Kong. Bottom row, leaf from top of flowering plant: G1, Varanasi-1; H, Hong Kong; 3, Varanasi-3. b, Comparison of *Xanthium* leaves under controlled conditions. Top row, left to right, developmental series, Hong Kong. Bottom row, leaf from top of flowering plant: A, Varanasi-4; H, Hong Kong; B, Bombay.

The Indian collections (Fig. 1a and b) with leaf and burr morphology similar but not identical to *chinense* plants of Louisiana⁷ showed a range of photoperiodic adaptation under 30–24° C (Table 1). The apparent critical nights of 10.25, 10.5 and 10.75 h are within the variance that Kaul¹⁰ described for the combined "monsoon" and "winter" forms of India.

Leaves from the 30–24° C programme were compared for isoenzyme patterns using gel electrophoresis¹¹. The Indian collections separated into two systems for peroxidase (Table 1) and for esterase. The *strumarium*-like plants of India and those from Hong Kong lacked bands that were present in the *chinense*-like plants of India and those of Louisiana.

Sesquiterpene lactones were examined in crude syrups of dried leaves using the techniques of Mabry¹² (Table 1). The nuclear magnetic resonance (NMR) spectrum for Louisiana plants indicated that the sesquiterpene lactone complement was almost entirely xanthumin¹³. The NMR spectrum of Hong Kong plants showed predominantly xanthinin¹⁴, although other sesquiterpene lactones were present. The Indian collections that resembled *strumarium* had xanthinin primarily. The spectra for various collections resembling *chinense* suggested intermediacy between the spectra for Hong Kong and Louisiana plants. The collection from Chandigarh contained xanthumin almost exclusively and had an NMR spectrum identical to that of Louisiana plants⁷.

The photoperiodic and chemical evidence suggests that *Xanthium* has become an aggressive weed in India as a result of the accidental introduction of the *chinense* complex from America and of its hybridization with the indigenous *strumarium* complex. Because the various morphological complexes are

Table 1 Morpho-physiology of *Xanthium* from India, Louisiana and Hong Kong

	Collection site	Latitude	Apparent critical night (h) *	Morphological complex †	Sesquiterpene lactones ‡	Peroxidase §
India						
	Chandigarh, Punjab	30° 44'	10.5	ch	M	—
	New Delhi, Delhi—1	28° 40'	10.25	ch	MX	A
	Varanasi, U.P.	25° 20'				
	Ganges R. banks—1		0	st	IX	—
	Ganges R. banks—2		0	st	—	—
	Ganges R. banks—3		10.75	ch	MX	—
	Banaras Hindu Univ.—4		10.5	ch	—	—
	Banaras Hindu Univ.—5		10.5	ch	—	—
	Bombay, Maharashtra	18° 56'	10.75	ch	MX	A
	Bangalore, Mysore	12° 58'				
	Lal Bagh—1		10.5	ch	MX	A
	Lal Bagh—2		0	st	IX	B
United States						
	Pecan Island, Louisiana	29° 45'	10.5	ch	M	A
Hong Kong						
	Hang Hau, New Territories	22° 27'	10	st	I	B

* Apparent critical nights for flowering were determined in varying night studies under 30° C day and 24° C night conditions. The night shorter than the one in which macroscopic floral buds were produced is indicated as the apparent critical night. The collections are burrs from a single plant except for Bombay, Chandigarh, Louisiana and Hong Kong.

† Symbols for morphological complexes: ch, closer to *chinense* in leaf and burr morphology; st, closer to *strumarium*.

‡ Symbols for sesquiterpene lactones: M, almost entirely xanthumin; MX, predominantly xanthumin; I, predominantly xanthinin; IX, predominantly xanthinin, but not exactly the same NMR spectrum as Hong Kong.

§ Symbols for peroxidase: A, system with more isoenzyme bands; B, system with fewer isoenzyme bands.

not separated by sterility barriers (McMillan, unpublished studies), hybridization between two complexes in the same area is likely. The introduced *chinense* complex is sensitive to photoperiod but the *strumarium* complex may be more sensitive to temperature than to photoperiod. Various combinations of temperature-photoperiod sensitivity resulting from hybridization may explain some of the diverse adaptations within the Indian populations. The Delhi plants with an apparent critical night of 10.25 h were least affected by temperature. They produced macroscopic buds at 30–24° C only two days earlier than at 24–15° C. Subsequent testing will explore temperature sensitivity in the *strumarium* complex.

The introduction of American *chinense* plants to India is not documented, but with the introduction of American cotton to India in the 1800s, burrs were probably accidentally imported. Louisiana *chinense* burrs covered by cotton fibres in the ginning process are difficult to clean and strongly resemble American cotton seed in size and shape. Indigenous Asiatic cottons do not have permanent fibre attachments to the seed as does American cotton. The *chinense* populations of Australia^{7,9} reportedly were a contaminant of American cotton seed shipped from India in the mid-1800s¹⁵.

The vast majority of the infestations of *Xanthium* which currently plague the subcontinent of India have greater morphological similarity to plants of southern Louisiana and the Caribbean islands than to plants indigenous to Asia. Because a high degree of aggressiveness is often characteristic of introduced plants, the niche capacities of *Xanthium* in India reflect the importation of traits that were new to the indigenous populations.

I thank Dr V. Kaul, Dr E. A. Ashby, Professor R. Misra, Dr P. S. Ramakrishnan, Professor S. C. Pandeya and Professor R. N. Singh for help and advice. I also thank Dr T. Mabry for the use of his NMR equipment and for discussions concerning sesquiterpene lactones. P. Chavez, N. Krause, E. Rodriguez and K. Walker assisted in chemical analyses. Travel funds were provided by the NSF Office of International Programmes.

C. McMILLAN

Department of Botany, and
Plant Ecology Research Laboratory,
University of Texas at Austin,
Austin, Texas 78712

Received July 3; revised August 11, 1972.

- Widder, F. J., in *Fedde: Repert. Spec. Nov. Regn. Veget.*, **20** (Berlin, 1923).
- Millsbaugh, C. F., and Sherff, E. E., *Field Mus. Nat. Hist. Publ.*, **204**, Bot. Ser., **4**, 9 (1919).
- Löve, D., and Dansereau, P., *Can. J. Bot.*, **37**, 173 (1959).
- Ray, P. M., and Alexander, W. E., *Amer. J. Bot.*, **53**, 806 (1966).
- McMillan, C., *Science*, **165**, 292 (1969).
- McMillan, C., *Amer. J. Bot.*, **57**, 881 (1970).
- McMillan, C., *Science*, **171**, 1029 (1971).
- McMillan, C., *Amer. J. Bot.* (in the press).
- McMillan, C., *Can. J. Bot.* (in the press).
- Kaul, V., *New Phytol.*, **70**, 799 (1971).
- Brewbaker, J. L., Upadhyay, M. D., Makinen, Y., and MacDonald, T., *Physiol. Plant.*, **21**, 930 (1968).
- Mabry, T., in *Phytochemical Phylogeny* (edit. by Harborne, J. B.) (Academic Press, London, 1970).
- Minato, H., and Horibe, I., *J. Chem. Soc.*, 7009 (1965).
- Geissman, T. A., Deuel, P., Bonde, E. K., and Addicott, F. A., *J. Amer. Chem. Soc.*, **76**, 685 (1954).
- Everist, S. L., *Queensl. Natur.*, **16**, 49 (1960).

Trends in the Evolution of Primate Mastication

A DETAILED study of feeding behaviour and tooth use has been made in four extant primates, *Tupaia*, *Galago*, *Saimiri*, and *Ateles*, using a combination of cinefluorography and occlusal analysis. These primates were chosen because they form a structural series: a parallel series of fossil primates, *Palenochtha*, *Pelycodus* and *Aegyptopithecus* has also been studied.

In primitive mammals^{1,2}, the cheek teeth are used both for ingestion and mastication, the incisors having a minimal function in food separation or trituration. This is also the case in *Tupaia* and *Galago*, but *Saimiri* and *Ateles* do use their spatulate incisors for biting all but the hardest food. This suggests that the shift from "ingestion-by-mastication"² in the canine-premolar and anterior molar tooth region to incisal biting is a specialization of higher primates associated with the development of spatulate incisors and, possibly, fusion of the mandibular symphysis. The symphysis is demonstrably mobile in the prosimians studied.

The action of the cheek teeth in ingestion-by-mastication is the same as that found in the first stages of mastication: the food is compressed between the approaching but not contacting occlusal surfaces of the cheek teeth. This action, "puncture-

crushing^{1,2}, produces characteristic wear on the tips of the cusps, flattening and, as can be seen in scanning electron-micrographs³⁻⁵, cavitating them after abrasion of the enamel. Once the food is adequately reduced, chewing follows. Examination of the molars in both series studied demonstrates that there has been a definite change in emphasis in their design from features associated with shearing or cutting^{4,5} to those associated with crushing and grinding. The duplication of upper shearing edges (*en echelon* shear⁴) typical of primitive tribosphenic molars, for example, is reduced in primitive primates (like *Palenochtha*) and progressively lost (for example, *Aegyptopithecus* and *Ateles*). Edges that are retained are orientated on the periphery of basins which are much expanded and provide grinding as well as crushing platforms. The net effect of these changes is to convert the molars from a cutting system with a minimal crushing action once they approach occlusion, to a system of pistons acting in sharp-edged compression chambers. The molars of *Aegyptopithecus* and *Ateles* are designed both to puncture-crush food in the preliminary stages of mastication, and to continue this activity, with the addition of some compartmentalizing shearing-grinding action, into the later stages when tooth-food-tooth contact is becoming tooth-tooth contact at the completion of the power stroke. The deeply gouged striations produced on wear facets during the power stroke demonstrate that in primates this movement involves a smooth change in its direction⁴.

At the beginning of a power stroke, the lower molar is being carried upwards, anteriorly and slightly medially, until the protocone reaches the bottom of the talonid basin (centric occlusion)¹ and then continues to move medially but more markedly anteriorly and now downwards so that the active side of the jaw moves towards the midline. The first of these movements is described as Phase I and the second as Phase II³⁻⁵. The relative distance traversed by the hypoconid of the lower molar across the upper in Phase I and Phase II can be measured and expressed as a ratio. Taking Phase II as unity, then the length of Phase I diminishes from 1.3 in *Tupaia* through 0.78 in *Galago* to 0.57 in *Saimiri* and *Ateles*, confirming the conclusions drawn from the change in tooth form.

The power stroke is the central component of the three stroke masticatory cycle. It is preceded by the preparatory stroke in which the mandible is moved upwards on the active side of the midline to bring the cheek teeth into buccal alignment in preparation for the power stroke. The recovery stroke follows which, in all four extant primates, involves an excursion across the midline towards the balancing side in the first half of the stroke, the return to the active side occurring in the second half. Although this shift towards the balancing side occurs, there is no evidence in the cinefluorographic recordings of a functional balancing side occlusion in any of the animals studied. A transitory contact may occur but there is no movement mirroring that on the active side as has been suggested by Mills^{6,7}. The terms "buccal phase" and "lingual phase" applied by Mills and descriptive of the occlusal relationships he found in Phase I and Phase II have therefore not been used.

Two distinct types of masticatory cycle are found in all four extant species. One is a puncture-crushing cycle, where no tooth contact occurs but which involves a greater vertical movement and smaller medio-lateral and antero-posterior components. The other is a chewing cycle where close or actual occlusal contact is reached and movement in preparatory and recovery strokes is often more exaggerated. Both cycles are rhythmically repeated, the number depending on the food consistency; an initial series of puncture-crushing is followed by chewing cycles until the bolus is passed.

The duration of each type of cycle and of its constituent strokes has been measured by using single frames from recordings taken at 60 frames a second⁴. The duration of each stroke is expressed as a percentage of the total cycle time. In overall cycles, *n* ranges from 93-111; in puncture-crushing cycles, *n* is 27-48 and in chewing cycles 63-66. The difference in stroke duration in puncture-crushing and chewing is highly

significant ($P < 0.001$), but there is no difference in the absolute duration of the total cycle with the exception of *Tupaia*. The puncture-crushing cycles in *Tupaia* are longer ($P < 0.05$) than the chewing cycles. In *Galago*, *Saimiri* and *Ateles* the puncture-crushing preparatory stroke is significantly longer ($P < 0.001$) and the recovery stroke shorter than in the chewing cycle; the power stroke is not affected. In *Tupaia* the power stroke is lengthened and the recovery stroke shortened.

The similarities in the time base for puncture-crushing and chewing cycles in the four species studied suggested there might be a common behaviour pattern in each type of mastication. The data for all puncture-crushing and chewing cycles were collected and examined using analysis of variance. This showed that puncture-crushing and chewing are highly significantly different types of behaviour. Each animal has its own variant of the basic puncture-crushing and chewing pattern, but this variation between the four different species is no greater than would be expected on a statistical basis for four animals of the same species. (The full analysis is currently in press⁴.)

Because the finding that the percentage duration of the power stroke does not change, although the proportion of its constituent phases does, we suggest that the observed changes in the morphology of the jaw apparatus in both series (*Tupaia-Galago-Saimiri-Ateles*; and *Palenochtha-Pelycodus-Aegyptopithecus*) have occurred within a behavioural framework established early in primate evolution, if not before^{1,2}.

We thank Dr R. W. Baker for help with statistical analysis and the support of the NIH USPHS and the Wenner-Gren Foundation.

K. HIIEMAE*

Department of Anatomy,
Guy's Hospital

R. F. KAY*

Department of Geology and Geophysics,
Yale University,
New Haven, Connecticut 06520

Received September 4, 1972.

* Also Museum of Comparative Zoology, Harvard University, Cambridge, Mass. 02138.

¹ Crompton, A. W., and Hiiemae, K. M., *J. Linn. Soc.*, **49**, 21 (1970).

² Hiiemae, K. M., and Crompton, A. W., in *Dental Morphology and Evolution* (edit. by Dahlberg, A. A.) (Univ. Chicago Press, Chicago, 1971).

³ Kay, R. F., and Hiiemae, K. M., *Mastication in Galago crassicaudatus*, *Prosimian Biology* (Duckworth, London, in the press).

⁴ Hiiemae, K. M., and Kay, R. F., *Evolutionary Trends in the Dynamics of Primate Mastication* (Karger, Basel, in the press).

⁵ Kay, R. F., and Hiiemae, K. M., *Tooth Usage and Mandibular Movements in Recent and Fossil Primates*, *Am. J. Phys. Anthropol.* (in the press).

⁶ Mills, J. R. E., *Dent. Practnr.*, **6**, 47 (1955).

⁷ Mills, J. R. E., *Archs. Oral Biol.*, **12**, 645 (1967).

Kammerer's Midwife

AS ANGSEESING¹ and other sources have revived this controversy, I wish to put forward a point that has so far not been mentioned but which may be fundamental.

The assumption of Kammerer, his critics and supporters, is that the female midwife toad tries to get away from the male, and has to be forcibly retained. This is *a priori* improbable, for the two animals are partners in reproduction. I have described the full sequence in four other Anuran species, and I have seen and heard *Alytes obstetricans* (midwife toad) at night around its native pond.

In the case of *Bufo bufo*² the male may tightly retain the female for hours or days. "Fighting" (attempts to dislodge the male by other males) is frequent, but when the female has

finished laying, she dismisses the male by a series of signals. The effect is dramatic. The male lets go and swims away in a few seconds. There is no struggle, and the female can repeat the signals if she is seized again.

In *Rana temporaria*² the female is retained by the male, sometimes for long periods. The eggs are all laid in a few seconds. The male then at once very quickly releases the female. If she is again seized, she croaks, and this signal, with her reduced girth, results in eventual release, even if on her way out of the pond she is seized by other males.

In *Bombina variegata*³ the female has a specific pattern for dismissing the male. It consists of mewing and crawling, which inhibits the male from persisting in attempts to retain her. She gets rid of the male with more difficulty, but without a struggle.

Females of *Xenopus laevis*⁴ sometimes get rid of the male by a quick twist of the body. This is usually, but not always, effective, partly because of their exceptional slipperiness and partly because the arms of the male are weak and the pads poorly developed. The male, but not the female, is entirely dependent on cutaneous respiration. Late in the night, dissolved oxygen falls, and amplexus cannot continue.

In each case, amplexus is not, as it appears, an iron grip by the male on an unwilling female, but is terminated automatically when it is no longer necessary. I consider that the real function of the pads is to enable a male to retain his female in spite of often violent attempts by other males to dislodge him. The pads are, in a way, weapons directed against other males.

If my limited observations of *A. obstetricans* are correct, there is no fighting and no need for pads, for the females probably seek out individual males, calling from scattered burrows around the pond.

Finally, the difficulty of breeding Amphibia by natural means in captivity is normally only overcome by painstaking attempts to simulate natural conditions. These conditions are often exacting and obscure. For example, *B. bombina* does not, in my hands, breed even if kept under the same conditions that induce full breeding in *B. variegata*. I doubt if attempts to breed *A. obstetricans* under unnatural conditions are likely to succeed, or have ever succeeded.

R. MAXWELL SAVAGE

Hill Top,
Roundabout Lane,
Welwyn, Hertfordshire

Received October 18, 1972.

¹ Angseesing, J. P. A., *Nature*, **239**, 408 (1972).

² Savage, R. M., *Proc. Zool. Soc.*, **55** (1934).

³ Savage, R. M., *Proc. Zool. Soc.*, **889** (1933).

⁴ Savage, R. M., *J. Zool. Lond.*, **165**, 245 (1971).

Oxidative Phosphorylation in Adult *Schistosoma mansoni*

THE blood fluke, *Schistosoma mansoni*, takes up large quantities of glucose and excretes lactic acid. It also uses oxygen but the role of this oxygen uptake in the metabolism of adult schistosomes is unclear. No increase in the rate of lactic acid excretion was observed when the worms were incubated under nitrogen and it was assumed that the flukes obtain all their energy by glycolysis¹. Further studies suggested that there were only sufficient cytochromes present to account for 1/10 of the oxygen uptake of the worms² and cyanine dyes inhibited 50% of the oxygen uptake without apparently adversely affecting them³.

Studies on the carbohydrate metabolism of larval *S. mansoni* demonstrated that when cercariae penetrate mouse skin there

was a switch from a fully aerobic metabolism involving cytochromes to a partially anaerobic metabolism where lactic acid was excreted and oxygen used⁴. Further experiments showed that at least part of the oxygen was used for energy production as a nitrogen gas phase increased the rate of glycolysis in the schistosomula. The same effect was observed with 3 day old schistosomula which had been cultured *in vitro* (G. C., unpublished results) by the method of Clegg and Smithers⁵. Because the metabolism of the young worms involved energy formation from glycolysis with excretion of lactic acid and from oxidative phosphorylation, adults were examined to see if they were similar to young worms.

There is some suggestion that adult worms are obligate aerobes. Worm pairs were cultured under 92% air, 8% CO₂ or 92% nitrogen, 8% CO₂ in Earle's lactalbumin and 10% newborn calf serum. The worms under nitrogen were more sluggish than those under air; pairs usually separated and produced no eggs. Worms under air produced a mean of 10 eggs per female over the first 3 days. Ross and Bueding had found worms survived longer aerobically than anaerobically *in vitro*⁶.

To examine the effects of atmospheres of nitrogen and oxygen on the metabolism of the worms, they were collected from sacrificed mice by perfusion with ice cold 'Tyrode' and were kept at 0° C until used. The effect of nitrogen on glycolysis rates was measured by gassing five worm pairs (7-8 weeks old) in 500 µl. 'Tyrode' containing 1 mg ml.⁻¹ of glucose with oxygen free nitrogen or air for 10 min at 0° C followed by 10 min incubation at 37° C. There was a consistent increase in lactic acid produced by worms kept under nitrogen, the ratio for lactic acid produced under air to that under nitrogen being 1:1.37 (±s.e. 0.01). About one quarter of the energy therefore is coming from oxidative phosphorylation. The figure is probably considerably higher *in vivo* because worm reproductive metabolism, as indicated by egg production, does not occur in 'Tyrode'. Similarly, incubation of worms in 2 × 10⁻⁴ M cyanide resulted in an increase in lactic acid production as observed under anaerobiosis.

To measure the effect of 2 × 10⁻⁴ M cyanide on oxygen uptake, adult worms were placed in a nylon basket in an oxygen (Clark) electrode at 37° C and, after the rate of oxygen use had been measured, cyanide was added. There was a 90% inhibition of oxygen uptake compared with 85% inhibition for freshly collected schistosomula and 83% for cercariae⁴. Ross and Bueding observed complete inhibition of oxygen uptake in worms cultured in 10⁻³ M cyanide⁶.

The inhibition of oxygen uptake by 2 × 10⁻⁴ M cyanide suggests the presence of functional cytochrome oxidase, but Bueding and Charms failed to find sufficient cytochromes to account for the schistosome oxygen uptake². Because cercariae are aerobic organisms and appear to have only normal cytochromes⁴, the cytochrome oxidase activity was compared in cercarial bodies and adults. Tails, which are lost on penetration, were removed by shaking cercariae at 0° C with glass beads and separated from the bodies by gravity sedimentation on a water 10% mannitol gradient. Cytochrome oxidase of washed particles was assayed at 30° C in the oxygen electrode in a pH 7.2 buffer (0.25 M glycerol, 10 mM KH₂PO₄, 5 mM MgCl₂, 20 mM Tris) with 0.5% defatted bovine serum albumin, 0.1 mM N-n-n' tetramethyl-para-phenylene diamine dihydrochloride (TM PD), 10 mM ascorbate and 0.07 mg ml.⁻¹ horse heart cytochrome C. The ratio of cytochrome oxidase activity per mg particulate protein in cercarial bodies, adult male and adult female worms was 1:0.8:1.3, and absolute determinations of activity suggested there was sufficient cytochrome oxidase activity to account for all the oxygen uptake of the adult worms.

To confirm the presence of functional oxidative phosphorylation a particulate preparation of adults was used as insufficient material was available to prepare pure mitochondria. Adult worm pairs were lightly homogenized in an all-glass homogenizer at 0° C in a sucrose-Tris buffer pH 7.2 (0.25 M sucrose,

10 mM KH_2PO_4 , 5 mM MgCl_2 , 20 mM Tris and 1 mM EDTA) and centrifuged at 2°C for 15 min at 10,000g. The pellet was washed twice and resuspended in the same buffer. There was a low rate of oxygen uptake by the particles which was stimulated by 10^{-2} M succinate or 10^{-2} M glutamate plus 10^{-3} M malate. There was little effect of ADP (1.8×10^{-4} M) on this preparation, but in the presence of 0.5% defatted bovine serum albumin ADP caused a marked stimulation of oxygen uptake. In the absence of ADP the respiration of the particles with succinate as substrate was stimulated by 2×10^{-4} M 2:4 dinitrophenol. In the presence of ADP and succinate the respiration of the particles was completely inhibited by 2×10^{-4} M cyanide and 10^{-6} M antimycin A. Using glutamate plus malate as substrate respiration was partly inhibited (59% reduction) by 10^{-3} M amytal and completely by 10^{-6} M rotenone. Worms, therefore, appear to possess a coupled functional cytochrome system.

The reduced coenzymes oxidized by the cytochrome pathway may be generated from two main sources. The first two enzymes of the pentose phosphate pathway have been assayed in schistosomes⁷, although the remaining enzymes have yet to be demonstrated. The use of inhibitors⁸ and the presence of some enzymes of the citric acid cycle, including threo-D₂-isocitrate: NADP oxidoreductase (1.1:1.42) and citrate oxaloacetate-lyase (4.1:3.7), (unpublished results, G. C.), suggests the worms possess a functional citric acid cycle.

The transformation of carbohydrate metabolism from the fully aerobic cercarial stage of *S. mansoni* to the parasitic stage in the mammal is thus only a partial transformation, and oxygen seems to be used in the adult for oxidative phosphorylation. Until a medium has been produced in which adult worm pairs lay eggs at the same rate as *in vivo* it will not be possible to determine the relative importance of glycolysis and oxidative phosphorylation in ATP formation.

I thank the Wellcome Trust and Overseas Development Administration for financial support.

G. C. COLES*

The Molteno Institute,
University of Cambridge,
Downing Street,
Cambridge CB2 3EE

Received August 2; revised September 13, 1972.

* Present address: Imperial Chemical Industries Ltd., Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire.

¹ Bueding, E., *J. Gen. Physiol.*, **33**, 475 (1950).

² Bueding, E., and Charms, B., *J. Biol. Chem.*, **196**, 615 (1952).

³ Bueding, E., Peters, L., Koletsky, S., and Moore, D. V., *Brit. J. Pharmacol.*, **8**, 15 (1953).

⁴ Coles, G. C., *Int. J. Parasit.*, **2**, 341 (1972).

⁵ Clegg, J. A., and Smithers, S. R., *Int. J. Parasit.*, **2**, 79 (1972).

⁶ Ross, O. A., and Bueding, E., *Proc. Soc. Exp. Biol. Med.*, **73**, 179 (1950).

⁷ Waitz, A. M., *Life Sci.*, **3**, 377 (1964).

⁸ Coles, G. C., *Comp. Biochem. Physiol.*, **34**, 213 (1970).

indications of purity. Variation in tapwater solutes makes it possible to distinguish samples by taste so that the same criterion may be true for purity variations in distilled water. This was tested here using once and twice distilled water.

Three subjects who were non-smokers were adapted to twice distilled water with once distilled water used as a stimulus and to once distilled water with a tapwater stimulus. A constant flow (330 ± 1 ml. min⁻¹) gravity system delivered adapting and stimulus solutions, at mouth temperature ($34 \pm 0.5^\circ\text{C}$), to the anterior dorsal surface of the tongue. When the subject was adapted, the stimulus solution was presented or the adapting solution continued. The signal detection rating procedure⁶, using a 6-point scale, was used to construct receiver operator characteristics (ROC) curves and calculate d' and nonparametric $P(A)$ values. 600 trials were performed for each subject at each adaptation level.

The tapwater was a typical sample⁷ from Bristol Waterworks, in the higher range for total hardness (160 – 228 p.p.m.), Cu (0.6 p.p.m.) and Pb (0.2 p.p.m.) with a low surfactant level. Once distilled water was produced in a 'Manesty Still' (type OBE), and twice distilled water was produced by redistillation in 'Pyrex' after passage through an 'Elgastat Cartridge C203' mixed bed ion exchange resin. The mean specific conductivity values for tap, once and twice distilled water were $24.6 (\pm 0.7) \times 10^{-6}$, $2.3 (\pm 0.1) \times 10^{-6}$ and $< 10^{-6}$ mho cm⁻¹, while the corresponding mean surface tensions were $72.0 (\pm 0.4)$, $71.4 (\pm 0.4)$, and > 71.5 dyne cm⁻¹. The polythene apparatus and storage vessels were cleaned with concentrated nitric acid and steamed until they showed minimal surface activity.

Signal strengths with the tapwater stimulus were as high as expected with $P(A)$ values of 0.99, 0.98 and 0.99. The second subject only had a realistic d' (3.0). The distilled water stimulus gave d' values of 2.53, 1.71 and 2.27 with corresponding $P(A)$ values of 0.96, 0.90 and 0.97 also indicating relatively easy detection. Complete adaptation was not always obtained, especially to twice distilled water, and was not necessary for signal detection. An initial trial period was also found necessary, confirming earlier studies⁸, which could be as many as fifty for the once distilled water stimulus, perhaps due to the difficult adaptation involved. Qualitative judgments were made and tapwater was described as "bitter" or "metallic" while once distilled water was described as "bitter", "metallic", "tingly" or "sweet". This reflects the inadequacy of a descriptive language for low signal levels.

Here I use once distilled and tapwater as supra-adapting stimuli for the first time. My results show that purity, as well as pre-adaptation, is an important factor in the distilled water taste.

M. O'MAHONY

Department of Psychology,
University of Bristol,
8-10 Berkeley Square,
Bristol BS8 1HH

Received April 5; revised July 10, 1972.

¹ Nagel, W. A., *Z. Psychol. Physiol. Sinnesorgane*, **10**, 235 (1896).

² Skramlik, E. von, *Handbuch der Physiologie der Niederen Sinne* (Georg Thieme, Leipzig, 1926).

³ Bartoshuk, L. M., McBurney, D. H., and Pfaffmann, C., *Science*, **143**, 967 (1964).

⁴ Bartoshuk, L. M., *Percept. Psychophys.*, **3**, 69 (1968).

⁵ McBurney, D. H., and Shick, T. R., *Percept. Psychophys.*, **10**, 249 (1971).

⁶ Green, D. M., and Swets, J. A., *Signal Detection Theory and Psychophysics* (John Wiley, London, 1966).

⁷ Barrow Treatment Works Average Analysis, Bristol Waterworks Co. (1970).

⁸ Linker, E., Moore, M. E., and Galanter, E., *J. Exp. Psy.*, **67**, 59 (1964).

Purity Effects and Distilled Water Taste

THE taste of distilled water has been shown to depend on previous adaptation¹⁻⁵. In studies where an exact description of purity is necessary, only the number of distillations are quoted, which is inadequate because purity varies with distillation efficiency. Measures such as specific conductivity for ionic impurities and surface tension for surfactants are better

Nomenclature for Rock-destroying Organisms

ROCK-DESTROYING organisms have interesting problems of adaptation¹ and are important agents in coastal erosion². The geologic significance of fossil rock-boring animals was recorded at an early stage³. Advances have been made in understanding the biochemical and biophysical mechanisms of rock erosion by some species^{4,5} and micro-morphological results of these processes have been described for a number of species^{6,7}. No universally accepted nomenclature has developed, however, which (1) separates all rock-destroying organisms from non rock-destroying organisms, (2) distinguishes those parts of the rock (surface or sub-surface) where the greatest modification takes place as a result of biological activity. Here I suggest a nomenclature.

Terms such as rock grazing, browsing, rasping, burrowing and boring are used to describe plants or animals that erode rocks, but no single term is applied to all rock-destroying organisms. Such a term should include reference to both "rock" as distinct from "sediment", and to "destruction" or "erosion" to distinguish those species that actually erode rock from those that nestle or take refuge in already formed "reliefs", but do not contribute any erosion themselves. These two criteria are satisfied by the term lithophagic used by Craig⁸ to refer to a freshwater snail responsible for erosion of limestone in southern British Honduras. It is now suggested that lithophagic (Greek: *lithos*, stone, rock; *phagos*, eating) be adopted and applied generally to any organism that erodes rock. Earlier terms such as lithodomous, lithotomous and lithophagous are by definition⁹ limited to burrowing and boring animals and exclude rock-grazing animals and rock-destroying plants (algae, fungi, mosses). The ecological term "cryptofauna" used by Evans¹⁰ is too broad and includes non-lithophagic as well as lithophagic species; some species may be lithophagic in some localities but non-lithophagic in others¹¹.

The nature and extent of rock destruction and the depth to which rocks are eroded by lithophagic organisms varies greatly and is partly determined by rock hardness¹² and other lithologic characteristics¹³. Three "vertical" zones are frequently distinguished in rock substrates which equate with the epifaunal, semi-infaunal and infaunal habitats normally used in reference to species located on or in sediments or soft substrates. Ginsburg¹⁴ defined three lithophagic forms on intertidal limestones in Florida: (a) grazing forms; (b) forms which have exposed, generally shallow burrows; (c) forms which penetrate the rock. Kühnelt¹⁵ introduced the following nomenclature for the same forms, (a) animals living on the rock surface occupy the *epilithion*, (b) those partially embedded the *mesolithion*, (c) those wholly embedded the *endolithion*. These terms have been used by Evans¹⁶ with reference to the lithophagic species *Penitella penita*, and their adoption is recommended. Using this nomenclature for communities it is clear that they include non-lithophagic as well as lithophagic species, that is those who occupy space created by lithophagic organisms but who do not contribute to rock erosion. This is also true of the recently introduced term "cryptobion" which refers to communities enclosed within living or dead coral which are revealed only by cracking the substratum open¹⁷. It is therefore necessary to distinguish between those organisms which have an active role from those which have a passive role.

I suggest the following nomenclature: (a) epilithophagic, (b) mesolithophagic, (c) endolithophagic. Epilithophagic species are lithophagic species inhabiting the epilithion; mesolithophagic species are lithophagic species inhabiting the mesolithion; and endolithophagic species are lithophagic species inhabiting the endolithion. Names for non-lithophagic species inhabiting the epilithion, mesolithion and endolithion are not proposed here. The precise boundaries in depth between the epi-, meso- and endolithion cannot be given at this stage.

A large number of terms are applied to the erosional results

of lithophagic species, such as pipes, perforations, galleries, tunnels, cavities, burrows and boreholes. In the absence of an all-embracing term it is suggested that biolithophagic "reliefs" should be used to cover all such forms developed in rock, following the terminology applied to biogenic sedimentary structures and trace fossils in sediments by Seilacher^{18,19}. Biolithophagic epireliefs, mesoreliefs and endoreliefs may be appropriate names for those reliefs identified in the epilithion, mesolithion and endolithion respectively. Further refinement of terms descriptive of the precise details of biolithophagic reliefs, such as their depths, widths, orientations, inclinations, shapes, sinuosities and geometries, is clearly required, but I do not attempt that here. Palaeontologists and paleoecologists in particular are put in a taxonomically difficult position because empty biolithophagic reliefs are often the only indications of the former presence of lithophagic organisms, and because modern taxonomy is based on soft-part anatomy of the organisms. I agree with Boekschoten's suggestion²⁰ that the best solution would be the creation of new names for either the holes or the organisms. There is merit in the former approach, but much more work on the description of biolithophagic reliefs needs to be done before this can be put into effect.

R. F. McLEAN

Department of Biogeography and Geomorphology,
Australian National University,
Canberra

Received July 18, 1972.

¹ Yonge, C. M., *Research*, **4**, 162 (1951).

² Jelu, J. T., *Scot. Geog. Mag.*, **34**, 1 (1918).

³ Barrows, A. L., *Geol. Soc. Amer. Bull.*, **28**, 965 (1917).

⁴ Otter, G. W., *Sci. Rep. Brit. Museum Nat. Hist.*, Great Barrier Reef Exped. 1928-29, **1**, 323 (1937).

⁵ Warburton, F. E., *Can. J. Zool.*, **36**, 555 (1958).

⁶ Hunter, W. R., *Proc. Roy. Soc. Edinburgh*, **63**, B, 271 (1949).

⁷ Evans, J. W., *Palaeogeog. Palaeoclimatol. Palaeoecol.*, **4**, 271 (1968).

⁸ Craig, A. K., *Science*, **158**, 795 (1967).

⁹ Howell, J. V., and Weller, J. M., *Glossary of Geology and Related Sciences*, second ed., 170 (Amer. Geol. Institute, Washington, 1960).

¹⁰ Evans, R. C., *J. Ecol.*, **37**, 120 (1949).

¹¹ Ebert, T. A., *Ecology*, **49**, 1075 (1968).

¹² McLean, R. F., *Bull. Mar. Sci.*, **17**, 551 (1967).

¹³ Neumann, A. C., *Limnol. Oceanog.*, **11**, 92 (1966).

¹⁴ Ginsburg, R. N., *Bull. Mar. Sci. Gulf Carib.*, **14**, 55 (1953).

¹⁵ Kühnelt, W., *Ann. Biol.*, **27**, 513 (1951).

¹⁶ Evans, J. W., *Veliger*, **10**, 148 (1967).

¹⁷ Morton, J. E., and Challis, D. A., *Phil. Trans. Roy. Soc. B*, **255**, 459 (1969).

¹⁸ Seilacher, A., in *Approaches to Paleocology* (edit. by Imbrie, J., and Newell, N. D.), 296 (Wiley, New York, 1964).

¹⁹ Seilacher, A., *Sedimentology*, **3**, 253 (1964).

²⁰ Boekschoten, G. J., *Palaeogeog. Palaeoclimatol. Palaeoecol.*, **2**, 333 (1966).

Hormones and Blood Chemistry

LAST year we had a unique opportunity to study the reprint request behaviour of scientists, as we published two papers almost simultaneously dealing with biochemical changes in human blood induced by synthetic steroid hormones. The first paper appeared in *Nature* as a single-page letter¹, while the second was a six-page contribution to a specialist journal². For one year we have collected and analysed reprint requests for these papers.

A total of 70 requests were received for paper 1 and 133 for paper 2 (writers asking for more than one reprint were classified as single requests). Despite the similarity of content between

the two papers, only three individuals requested both; so that we received communications on the two papers from exactly 200 scientists. There were 8 requests with insufficient postage, which we refused to accept, and 6 requests for papers we had not written in journals to which we have never contributed.

Our papers 1 and 2 appeared in "Current Contents" for September 1 and August 11, 1971, respectively, and in "Chemical Abstracts" for December 6 and September 27, 1971. Our first investigation was to analyse requests for each paper by date of posting (Table 1). Only 7 individual requests out of 188 communications with legible dates were posted before publication of the titles in "Current Contents", but over 70% of requests were posted before the appropriate issues of "Chemical Abstracts".

Table 1 Postal Dates of Requests

Paper No.	Total requests	Requests with legible date		Requests per month (% legible) weeks:			
		No.	% of total	0-4	5-9	10-14	15-19
1	70	65	93	9	35	30	13
2	133	123	92	c. 1	45	33	12

The contents page of *Nature* allows a test of how many requesters read the original paper 1, for titles on this page (which is reproduced by "Current Contents") are not those of the published papers. Of the 70 requests for paper 1, only 46 asked for it by title (the remainder gave only the journal reference), and 39 of these (85%) used the title found in "Current Contents", not that heading the actual paper. We were able to apply a similar test to paper 2 by examining the address used by our requesters. In the pages of the journal, the box number was included in our address, but in "Current Contents" it was omitted. Of 133 requests for paper 2, only 4 included our box number in the address on their cards.

We analysed our requests by country of origin and type of institution (Table 2). Approximately half the requests for both papers came from America, but none came from Russia or China.

Table 2 Requests by Country and Institutions

Country	% of total		% of country's requests		
	Paper 1	Paper 2	University	Hospital	Research institute
USA	47	51	48	28	19
Czechoslovakia	9	2	80	10	10
Canada	7	9	40	50	10
UK	6	5	55	45	0
GFR	4	7	30	50	20
France	6	5	20	30	40
Others*	21	21	65	25	10

* Austria, Australia, Argentina, Brazil, Chile, Costa Rica, DDR, Hungary, India, Israel, Italy, Netherlands, Norway and Sweden.

If our findings are typical, the average reprint-requester is American, works in a university, requests publications without having seen the original, and is able to afford airmail postage rates. We also note that his post office is uncertain of rates to central Africa, for almost a third of our requests are incorrectly stamped.

This brings out the central paradox of reprint requesting: our papers could have been photocopied for less than the cost of sending a reprint request. We are unable to formulate any satisfactory hypothesis of motivation at the present, but, in

the hope of gathering more information, we have given this letter a deliberately misleading title.

M. H. BRIGGS
M. BRIGGS

*Department of Biochemistry,
University of Zambia,
PO Box 2379, Lusaka,
Zambia*

Received September 22; revised October 12, 1972.

¹ Briggs, M. H., Briggs, M., and Austin, J., *Nature*, **232**, 480 (1971).

² Briggs, M. H., and Briggs, M., *Contraception*, **3**, 381 (1971).

GENERAL

On Boiling an Egg

It has long been accepted that on top of Pike's Peak, where water boils at 91° C, it takes 12 h to hard boil an egg. We have conducted experiments in the Biochemistry Department, Oxford University, in which we have hard boiled eggs successfully at 91° C in 10 min. There would thus appear to be a large error between the predicted and the experimental times.

By heating eggs for definite periods of time at various temperatures between 95° C and 65° C we have been able to estimate the relevant activation energies for denaturation of the yolk and albumin. These are ~11 kcal mol⁻¹ and ~24 kcal mol⁻¹ respectively. The method involves comparing the fluidities of the albumin and the hardness of both the albumin and yolk at the various temperatures. These figures should be compared with the previously accepted value of ~130 kcal mol⁻¹ (ref. 1).

At 71° C after 10 min the hardness of the yolk is similar to that after 3 min at 91° C but the albumin remains very fluid even after 30 min. This could have important applications in cookery.

R. A. DWEK

*Department of Biochemistry,
University of Oxford*

G. NAVON

*Department of Chemistry,
Tel Aviv University,
Tel Aviv*

Received September 8, 1972.

¹ Moore, W. J., *Physical Chemistry*, fourth ed., 300 (Longmans Green, London, 1965).

Recent Art Forgeries: Detection by Carbon-14 Measurements

THE faking of paintings is centuries old. Respectable imitation in the 15th and 16th centuries was followed by more profitable and now flourishing forgery¹. Although sophisticated scientific techniques have been applied to problems of authentication², most of these methods are concerned with the detection of forgeries of works 200 or more years old. The present trend, however, is to forge works of the late 19th century. There are some obvious advantages from the forgers' point of view: artists' materials typical of the period are still available, the production of work in a modern style is easier and less time consuming because very great age is troublesome to fake and, because prices are lower, less care is taken in examination and analysis before purchase.

We have investigated the detection of increased concentrations of ^{14}C in artists' materials to identify works of art produced since the advent of atmospheric nuclear weapons testing. The use of conventional ^{14}C dating to determine the age of paintings is prevented by the short timescale involved and by the need for large samples. The very large increase^{4,5} of atmospheric ^{14}C caused by nuclear weapons testing in the 1950s and 1960s does, however, offer a means of identifying substances produced from materials that were biologically active during and since that period.

Linseed oil and other drying oils were used extensively as media for pre-World War II paintings and are used for forgeries of such paintings. Linseed oil has a fairly short shelf life, and significant quantities of old material are not available; we therefore assumed that this would be a likely material in which to seek man-made ^{14}C . We obtained specimens of linseed oil dating back to 1945 and measured the ^{14}C concentration by liquid scintillation counting.

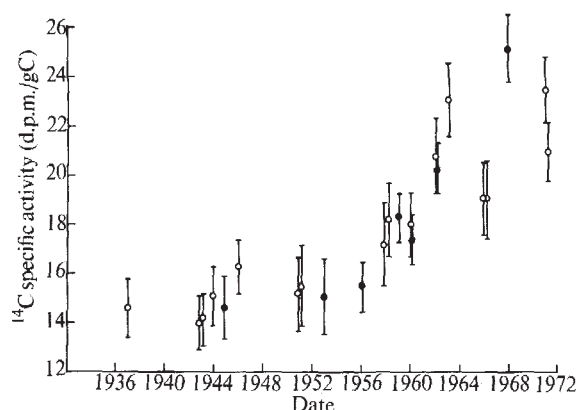


Fig. 1 The ^{14}C concentration in linseed oil and in paper as a function of date of production or of use, respectively. ●, Linseed oil; ○, paper. Indicated uncertainties (σ) are due mainly to counting statistics.

The samples were decolourized by diluting with diethyl ether and treating the solution with activated charcoal. The ether was then removed by vacuum drying followed by drying under argon at reduced pressure at 60°C . A weighed portion of the decolourized oil was dissolved directly in a toluene-based scintillator and the radioactivity was measured. Aliquots of a ^{14}C standard solution were added and the radioactivity measurement repeated to correct for quenching effects. The results, shown as dark circles in Fig. 1, showed the expected increase in ^{14}C concentration, nearly doubling in the period 1945 to 1968.

Our results show that it appeared possible to detect such differences in the oil from a sample of paint as small as 100 mg (assuming 50% linseed oil in the paint). To measure the expected low radioactivity (approximately 0.6 to 1.2 disintegrations per minute) in such small samples (less than 40 mg carbon) a gas proportional counter operated in anti-coincidence with a guard detector is required. Accordingly we constructed such a system⁶ with a detector designed to hold 17 mg of carbon as carbon dioxide at a pressure of one atmosphere. The system had a ^{14}C counting efficiency of 40% and a background of approximately 0.4 c.p.m. Samples were prepared by conventional means.

We used this system to make repeated measurements on small (50–100 mg) samples of linseed oil produced in 1952, 1962, and 1968 and on two paint samples taken from paintings dated 1933 and 1966. The latter samples weighed 100 and 120 mg, respectively. Allowances should be made for any pigment present that might also be a source of carbon. For example, pre-treatment with an acid could eliminate interferences from carbonates such as lead white. The results obtained are shown

Table 1 ^{14}C Measurements in Small Samples of Linseed Oil

Sample date and type	^{14}C specific activity [d/m/gC (σ)]
1968 (oil)	32 ± 5 (average of 4 determinations)
1962 (oil)	21 ± 3
1952 (oil)	17 ± 4 (average of 4 determinations)
1966 (painting)	35 ± 5
1933 (painting)	19 ± 4

in Table 1. These data demonstrate that one can distinguish oil paintings produced prior to the early 1950s from those produced in the late 1960s.

While forgers conceivably could acquire older specimens of paper, wood, and canvas, the supply is limited and hence the detection of man-made ^{14}C in these materials is also of interest. The analysis of paper is of particular importance because of the frequent occurrence of forged drawings and watercolours. We assumed that a one gram sample of such materials may usually be obtained and, hence, that relatively simple liquid scintillation counting could be used for the measurements. Undoubtedly the use of a proportional counting system such as those used for conventional ^{14}C dating would yield more precise results.

We devised a method⁶ in which the carbon dioxide, obtained from combustion of the sample, was sealed under pressure in a quartz tube containing a toluene-based, liquid scintillator of conventional formulation. This method yielded an overall efficiency, for preparation and counting, of approximately 50%. We used the method to analyse a total of 16 samples of paper representing 11 dates (of usage) ranging from 1937 to 1971. Duplicate determinations were made on five of the specimens.

The results, shown as open circles in Fig. 1, indicate that paper of the 1960s can be distinguished from that produced prior to the 1950s. The increase in ^{14}C concentration corresponds to that observed for linseed oil. However, occasionally a modern sample may be encountered which falls below the expected curve because the whole tree is used for papermaking, a substantial part of which could have grown before the "nuclear age".

We conclude that the detection of high concentrations of ^{14}C in artists' materials offers an objective means of detecting recent art forgeries.

This work was supported jointly by the National Gallery of Art (Washington) and the Division of Applied Technology of the United States Atomic Energy Commission. We thank D. K. Forstad of Spencer Kellogg Division of Textron, J. C. Cowan of the United States Department of Agriculture, L. V. Anderson of the Minnesota Linseed Oil Company, and E. C. Hulmer for samples and information on the production and composition of linseed oil.

B. KEISCH
HOLLY H. MILLER

Division of Sponsored Research,
Mellon Institute, Carnegie-Mellon University,
Pittsburgh, Pennsylvania 15213

Received June 12; revised August 1, 1972.

- Kurz, O., *Fakes*, 31 (Faber, London, 1958).
- Miller, F. J., Sayre, E. V., and Keisch, B., *Isotopic Methods of Examination and Authentication in Art and Archaeology* (Isotope Information Center, Report No. ORNL-IIC-21, Oak Ridge, 1970).
- Keisch, B., *Stud. Conserv.*, **15**, 1 (1970).
- Libby, W., Berger, R., Mead, J. F., Alexander, G. V., and Ross, J. F., *Science*, **146**, 1170 (1964).
- Rafter, T. A., *NZ J. Sci.*, **8**, 472 (1965).
- Keisch, B., and Miller, H. H., *Application of Nuclear Technology to Art Identification Problems—Third Annual Report*, 30 (US Atomic Energy Commission Report No. NYO-3953-3, Washington, DC, 1971).
- Keisch, B., and Miller, H. H., *Application of Nuclear Technology to Art Identification—Final Report*, 17 (US Atomic Energy Commission Report No. COO-3034-2, Washington, DC, 1972).

BOOK REVIEWS

Sense and Sensibility

Sterne und Menschen. By Albrecht Unsöld. Pp. viii+170. (Springer: Berlin and New York, 1972.) 29.80 DM; \$9.30.

In thirteen short essays one of the world's leading astrophysicists conveys his thoughts upon many facets of his science and its creators. The individual pieces are dedicated to diverse scientific occasions during the past two decades; there is no connexion between them save the involvement of the author, and one of the rewards for the reader is that they make him much better acquainted with the author himself. For Professor Unsöld has long been a legendary figure in astrophysics, famous for his work on stellar atmospheres and related problems of the abundances of the elements, and for his monumental *Physik der Sternatmosphären*; and more recently, by his book *Der neue Kosmos* and its translations into English and other languages, he has gained fresh fame as an expositor of modern astronomy as a whole; now in *Sterne und Menschen* he reveals himself as the possessor of an even greater range of talents which he exercises with rare sensibility.

Here Unsöld writes with the meticulous scholarship of his other works, but with a lighter touch appropriate to such occasional essays. For instance, in one we meet Heinrich Hertz *not* making his most famous discoveries in Kiel because the winters there made him so miserable; in the next we learn how far Hertz went in anticipating certain features of general relativity some 20 years before the work of Einstein; in another, Unsöld can in a few sentences conjure up the intellectual climate of the 19th century forbears of his contemporary astronomer Paul ten Bruggencate.

Several of the items arise out of Unsöld's 40 years of devoted service to the University of Kiel. Indeed, the first is his Rectorial address of 1958 in which he gives a masterly survey of the basic ideas of physics and their place in the whole of human thought; those about Max Planck, Heinrich Hertz and Walter Kossel are occasioned

by associations they had had with Kiel, and so on. There are also acute notices of the astronomers Otto Struve and M. G. J. Minnaert. Fortunately the set includes two addresses in which Unsöld expounds in a general way his own views about the abundances of the chemical elements and their origins—the outcome of a lifetime of thought upon the subject. The book will rank amongst those that earn for science its place among the humanities.

W. H. McCREA

Biological Rhythms

The Living Clocks. By Ritchie Ward. Pp. 319. (William Collins: London, March 1972.) £2.50.

THIS book is more nearly a collection of about twenty independent short stories, all of them interesting and lively, none requiring sustained attention. Thus it can be fitted into a pleasant evening in and out of your favourite chair at home.

Ward relates in historical and biographical format the remarkable observations of naturalists and laboratory biologists interested in daily rhythms. His prose is lively and sustains attention, though frequently lapsing into uncritical excesses of enthusiasm, for example when exclaiming that the discovery of internal rhythms is at least as profound as Einstein's paper of 1905 and as glamorous as Watson and Crick's unravelling of the physical basis of heredity. Off-putting as such hyperbole may be, the fact should not be overlooked that some astonishing facts are reviewed in these vignettes, almost all of which await explanation.

Introductory chapters mine the little-known literature of daily and seasonal rhythms, bringing to attention such gems as the entry in Columbus's logbook that flickering lights were sighted from the Santa Maria in the dark West Indian seas at exactly the hour and phase of the Moon when, we now know, the Bermuda fireworm discharges gametes in luminous streams.

About one-third of the way through, Ward's style becomes more biographical

and more authoritative, benefiting from the personal interviews he arranged with prominent "clocks" investigators, from the venerable pioneers down to a cautious cutoff age of about forty. Ward makes no attempt to segregate the prophets from the heretics, though he is careful to point out that some particularly spectacular claims are not widely believed. His few errors of fact, omission, or scientific precedent are, I think, mainly of interest to scholars involved professionally and need not distract from the central message: that the most diverse organisms—maybe even all eukaryotes—really are organized around almost-daily, -tidal, and -seasonal periods, occasionally with startling persistence and precision such as we see in the navigational feats of migratory birds, for example . . . and that these phenomena were encountered in surprising ways by interesting people. Ward points to vaguely foreseen implications for agriculture, ecology, and medicine, such as the possibility of minimizing "jet-lag" by exposing travelers to a well-timed light pulse, or exterminating crop pests by aborting their winter diapause with field lights.

The physiology underlying these phenomena remains as obscure as the names of the younger scientists now seeking ways to enquire more deeply into them. I anticipate that the correlation between these two omissions will become manifest within ten years.

A. WINFREE

Agrarian Britain

Patriotism with Profit: British Agricultural Societies in the Eighteenth and Nineteenth Centuries. By Kenneth Hudson. Pp. xii+143. (Hugh Evelyn: London, 1972.) £5.25.

THE object of this book, says the author in his introduction, is to eschew the Great Man interpretation of English agrarian history, typified by Lord Ernle in his praise of the traditional agricultural heroes, Townshend, Bakewell, and Coke, in favour of a social analysis of agricultural progress. Specifically, he wishes to demonstrate that the

pioneering work of local farming societies paved the way for the Royal Agricultural Society (founded 1838), and that they "were an indispensable element in the process of making a highly conservative industry change its habits and beliefs. . . ." As it turns out, the strength of the book proves to be its almost antiquarian love of detail, rather than its attempt at historical and sociological analysis. Through the use of numerous well-chosen photographs and lengthy descriptions of (for example) agricultural shows, the author does re-create for us the social flavour of agrarian society—certainly a worthwhile achievement. In terms of its intended goals, however, the book is far less successful. History is still seen as the product of a few powerful personalities, and the societies themselves, from Hudson's description, appear to be active and even entertaining, but possibly innocuous.

Part of the problem derives from the too selective use of sources. There were roughly eighty agricultural societies in 1810, yet Hudson relies for his evidence on the publications of a very few, and most heavily on those of the Bath and West of England Society. The Board of Agriculture, for example, the logical ancestor of the Royal Agricultural Society, is barely discussed, and its *Communications* not cited at all. But even given the context of a restricted survey, the essential argument—that these societies made an actual difference for farming practice—is not convincingly made. All of the societies were primarily concerned with the "diffusion of useful knowledge", frequently via published reports, but as Hudson tells us in the final chapter, a high proportion of farm workers were illiterate. Courses were sometimes held, with "mechanics" admitted gratis, but how many actually attended, let alone benefited? Medals and premiums were offered for needed improvements, but we are not given any figures regarding numbers of awards made. The author reveals his own ambivalence on this point when he notes that the societies had as their most important object making farming businesslike, but adds: "How much success the campaign had it is impossible to say." This is precisely the point: the problem Hudson poses may be one that by its very nature admits of no satisfactory solution.

What, then, was the function of these societies? In what appears to be a different approach, the author argues that they "were in fact microcosms of the British ruling class and as such functioned to some extent as agents of social control". This is sound Marxism, and there is much historical evidence to bear the thesis out. Surely such organizations can be seen, at least in

part, as attempts to cope with the problems caused by the massive enclosures of 1760–1815, the fear generated by the French Revolution, and the agrarian riots that racked England during 1795–1830. Yet Hudson chooses not to cite these events, and in fact does not even proceed to develop the argument. He does mention the allotment programmes, by which the rural labourer was to be made self-supporting (given his own cottage, for example), but considering their almost total failure it becomes hard to see how they contributed to social control.

In the absence of a coherent social interpretation of the significance of these agricultural groups, the author ultimately reverts to Great Man history. He tells us that the successful societies were the work of a very few men "of exceptional enterprise and public spirit", whose goal was "to pump new life into the countryside. . . ." These "dynamic rural father-figures" succeeded "by sheer determination and strength of personality", and regarded "their local agricultural societies [as] a very useful if not essential tool" for furthering their aims. Without such men, concludes Hudson, "it is doubtful if either the Bath and West [of England Society] or any other provincial society would have achieved a great deal". But what was actually achieved is never made clear, and how it was achieved, on this final interpretation, would have drawn the approval not only of Lord Ernle, but also of Samuel Smiles.

Despite these drawbacks, the book is a valuable source of new information, and in particular has much to offer the historian of science. In his examination of the sponsorship of scientific activity by these societies, Hudson reveals that the popular conception of science in the early nineteenth century was frequently agricultural. Chemical analyses and lectures were viewed as a natural part of a society's programme. The Dublin Society, for example, appointed William Higgins Professor of Chemistry and Mineralogy, set up a laboratory, and hosted lectures on botany and experimental philosophy. Higgins's successor, Edmund Davy, managed to discover acetylene in between his investigations of potato starch. The Royal Agricultural Society itself had as one of its stated aims to "encourage men of science in their attention to the improvement of agricultural implements, the construction of farm buildings and cottages . . . and so on". It is remarkable that as late as 1838, with the trend toward scientific specialization and professionalization in full swing, ploughs and cottages were still viewed by England's most enlightened citizens as proper subjects for "scientific" investigation. If organized support of scientific research

did receive its first major impetus from agricultural societies, the social history of English science during the early years of the Industrial Revolution will have to be carefully re-examined. By demonstrating the existence of a heavy overlap between the scientific and agricultural communities, the author has performed a valuable service.

MORRIS BERMAN

Hall Effect

The Hall Effect in Metals and Alloys. By Colin Hurd. Pp. xiii+400. (Plenum: New York and London, 1972.) \$23.

THIS book is one of the International Cryogenics Monograph Series, and follows a related volume on the electrical resistance of metals by G. T. Meaden. The first part consists of five chapters on the general theory of the Hall effect and one chapter on experimental methods; the second part is a comprehensive review of the literature on metallic elements and binary metallic alloys.

The first chapter defines the Hall effect, and describes in a clear, although largely qualitative, manner the dynamics of electrons in metals at low temperatures; the second chapter describes various limiting cases. The third chapter is a brief description of the Hall effect in nearly free electron metals designed to provide a conceptual basis for the following two chapters: the first of these is concerned with Group 1B metals, and the second with magnetic metals.

In a book of this kind it is difficult to decide whether to produce an essentially self-contained handbook, or to provide a broad survey supplemented with copious references. Dr Hurd has chosen the latter method, so that (for example) it would not be possible for a newcomer to the field to construct a Hall effect apparatus on the basis of the sixth chapter in this book alone. For the same reason he has concentrated on a largely qualitative approach to the various topics covered, concentrating on physical understanding. In places this may possibly have been overdone: only ten pages are given to a discussion of the theory of the Hall effect in the low field limit; the calculations of Ziman and of Dugdale and Firth for the Group 1B metals are described very briefly and qualitatively; there is very little discussion of the situation in non-cubic metals. These are, however, minor criticisms.

The book is very well presented, and the qualitative approach and the author's clear style make it very easy to read; the comprehensive survey of the literature will make it indispensable to anyone working in the field. Un-

fortunately, the price is rather high, and this may put it beyond the reach of research students who would find it especially useful. JOHN STRINGER

Sedimentology

Origin of Sedimentary Rocks. By H. Blatt, G. Middleton and R. Murray. Pp. xix+634. (Prentice Hall: Englewood Cliffs, New Jersey, July 1972.) \$16.

SOMEONE had to try to summarize and evaluate the many advances in sedimentology—in data, techniques and ideas—that lie buried in the post-Pettijohn¹ plethora of literature. I think the authors have succeeded—though one reviewer can hardly hope to be so informed he can assess adequately that which has taken three authors to put together.

The book consists of twenty chapters divided between six parts. Part 1 (one chapter) outlines the aims and methods of study of sediments. Part 2 (five chapters) discusses the physics of sedimentary processes and deals with the geologic cycle, sedimentary textures, sediment movement by fluid flow, sedimentary structures and facies models. Parts 3, 4 and 5 (thirteen chapters) cover the mineral and chemical composition, classification, origin and diagenesis of the common (*sic*) sedimentary rocks. The final chapter (Part 6) outlines current thinking on the major external controls of sedimentation.

The material (inevitably uneven and with the occasional factual error) is in the main treated in an informative and enjoyable manner, with insight, conviction and, on occasion, humour. Most important, the authors are not afraid to make value judgments and they are particularly generous with their suggestions for future lines of research.

My main criticism concerns what has been left out—perhaps because these are the rocks with which I am most familiar! Surely one would expect sections on coal, lignite and oil-shale? Sedimentary rocks containing metals are also virtually ignored. There is nothing, for example, on the Colorado Plateau uranium-copper-vanadium province, or on metalliferous black shales. No mention is made of manganese ores in chapter 19, in spite of its title—and so I could go on. Why do geologists (not just sedimentologists) write petrological texts that ignore so many rocks coming within their terms of reference apparently solely because they are of economic value? It is scientifically unsound and potentially dangerous. Future geologists must be made to realize that a study of “economic”

rocks is no different from a study of rocks of “academic” interest, otherwise there is little hope of our finding new sources of supply of the materials necessary to maintain our civilization.

In spite of this criticism I have no doubt that the book will stimulate the next generation of sedimentologists and take its place as the obligatory text in most university courses. To the practising geologist intent on catching up with many aspects of sedimentology he has fallen behind with, there is no rival. It is beautifully produced (with the occasional exception of some photographs) and free from misprints (though I suspect the inclusion of references in bold type in the title of Section 4.7 is an editorial lapse).

P. McL. D. DUFF

¹Pettijohn, F. J., *Sedimentary Rocks* (Harper and Row, New York, 1957).

Needle and Haystack

Atomic Safeguards: A Study in International Verification. By Allan McKnight. Pp. xxii+301. (United Nations Institute for Training and Research, New York, 1971.) \$6.50.

THE nuclear fuels which fire up modern electric plants are schizophrenic materials. In dilute form they suffice to sustain a controlled chain reaction as an essential source of energy for peacetime. In concentrated form they may be adapted for use in nuclear explosives. The “atoms for peace” made famous by President Eisenhower’s 1953 speech to the United Nations have a dual identity and it becomes the task of modern society to keep nuclear material confined to the fuel cycle of peacetime industry.

An amount of concentrated or fairly pure nuclear material about the size of a baseball is adequate for a low-power nuclear explosive. But as nuclear power grows from its present infancy to more maturity the world’s power plants will consume huge quantities of nuclear fuel. As a result more than a thousand tons of potential bomb-stuff will be circulating in the nuclear fuel cycle before the end of the century. Keeping track of this precious and precocious stuff is increasingly a needle-in-the-haystack proposition with the haystack getting bigger every year.

The problem of this nuclear needle-hunting forms the basis of *Atomic Safeguards*, written by Allan McKnight, the first Inspector General of the International Atomic Energy Agency (IAEA). No agreement such as the Non-Proliferation Treaty will be worth much if there is no inspection system devised and constantly implemented to keep account of the nuclear material as it goes into nuclear power plants, comes out as spent fuel, enters storage tanks,

undergoes reprocessing to recover unburned nuclear fuel and is refabricated as fuel elements. In some parts of the fuel cycle, radioactivity becomes a protective device since diversion of it becomes unwieldy due to the sheer weight of shielding required to handle it safely. Slightly enriched nuclear fuel, while not such a hazard, is not a candidate for basement bomb-making. The point of greatest vulnerability in the fuel cycle is the plutonium recovery and, in the future, in the power-breeder fuel which will be 15 or more per cent plutonium oxide.

Atomic Safeguards is a comprehensive and competent examination of this troublesome nuclear issue with emphasis on (Part I) the political development of IAEA safeguards and (Part II) the executive administration of these safeguards. Half of the book consists of a series of nine annexes which back up the text and provide details of pertinent treaties and technologies.

Given the escalation of fanatical extremism in the world it becomes necessary to worry about the siphoning off of nuclear material to groups which might convert it into a nuclear weapon. It requires little imagination to glimpse the potential mischief in store for society when some vulnerable part of the social anatomy is threatened with a blackmail or retaliatory coup. The UNITAR’s (UN Institute for Training and Research) rather viscous prose tends to obscure the contemplation of the consequences as it focuses on administrative and inspection mechanisms. But the magnitude and intensiveness of the study testify to the significance which UN officials attach to the nuclear safeguards problem.

The shape of the future of nuclear power is still hard to discern. To be sure, we know approximately how many and what size power plants will come on stream decade by decade. The world’s rapidly depleting premium fossil fuels guarantee a world rush to heavy metal energy. About the only event capable of arresting the momentum of this juggernaut would be a serious accident, but even this would not stop nuclear power but would mean an interruption. But where these plants will be sited and how they will be clustered and what pattern the fuel network will assume is still uncertain. One possibility which offers hope for increased safeguards against illicit acquisition of nuclear material is the evolution of self-contained reprocessing centres where the input is spent fuel and the output is fresh fuel. Such a development could serve to concentrate the circulation of high grade plutonium within tightly controlled security boundaries.

The UNITAR study, a solid contribution to the nuclear safeguards problem, is essentially an administrative introduc-

tion to the new art of nuclear snooping—the fine tooth-combing of the nuclear haystack to make sure that potential bomb-stuff does not fall into a black market.

RALPH LAPP

Crystallographic History

Early Papers on Diffraction of X-rays by Crystals. Edited by J. M. Bijvoet, W. G. Burgers and G. Hagg. Volume II. Pp. xix+484. (A. Oosthoek's Uitgeverijmaatschappij: Utrecht, 1972.) (Published for the International Union of Crystallography.) £10.80.

THE two volumes of this collection of papers cover the period from the birth of the subject in 1912 to the year 1935; but the papers are not in chronological order: they are arranged in such a way as to display the development of each facet of the subject separately. Volume I, published in 1969, contained the papers in which, following the initial discoveries by von Laue and the Braggs, the theoretical foundations were laid, the first crystal structures were revealed, and the analysis of the several factors affecting the intensities of diffracted beams put crystal structure determination on a firm quantitative basis. Volume II covers the rich harvest of structures which grew from these firm foundations, and the growing knowledge of atomic radii and building principles during the twenties and early thirties.

The papers, not always in complete form but sometimes represented by an excerpt, a paragraph or only a few sentences, are given in the original languages; they are grouped in sections, each covering a particular facet, ranging from space groups and building principles to experimental methods and the various types of structures of increasing complexity which were successfully revealed in this period by the indirect puzzle-solving trial method. The book ends with Patterson's paper of 1935 which, by showing that a vector distribution can be calculated unambiguously from the X-ray data, inaugurated the era of semi-direct methods. The development of the subject is displayed admirably, not only by this arrangement in sections but also by page headings which give on the left the authors and on the right the topics dealt with in the open pair of pages.

A slightly inconvenient feature is that each contribution is preceded only by a number; the author and subject can be gathered from the page headings, but to find the date we have to refer back to the beginning of the section, while the journal reference is given only in the contents list at the beginning of the book. I noticed a few typographical errors, the most serious of which are dates with figures transposed, in the

lists of contributions at the beginnings of sections; however, the dates are given correctly in the contents list at the beginning of the book.

But these are small blemishes in an admirable production. All crystallographers interested in the history of their subject will find these volumes of enormous interest; the editors have performed a most valuable service in arranging the significant contributions in this way. The experienced worker can savour again the drama and excitement of the early days, or perhaps (I speak here for myself) can fill in gaps and correct his perspective of the development of the subject; and the teacher will appreciate the convenience of having the early documents of the subject collected together in this way. As for the student, the book is intended "not only to serve as a pedagogic aid, but also to make the student aware of the history of his science". The reviewer of Volume I (*Nature*, **224**, 984; 1969) was doubtful of its pedagogic value, but I would urge that students should be encouraged not to rely only on textbooks but to go to original papers to get the feel of fundamental research, the struggle to understand novel phenomena. Lastly, the historians and philosophers of science will find that, read in conjunction with the personal reminiscences of the chief protagonists collected together in 1962 by Ewald in *Fifty Years of X-ray Diffraction*, these volumes will provide most of what they want to know about the early development of this subject which has played such a crucial role in twentieth century science.

C. W. BUNN

Blood

Modern Concepts in Hematology. Edited by G. Izak and S. M. Lewis. Pp. 278. (Academic: New York and London, August 1972.) \$26.

THIS book gives an account of views expressed by four invited panels of the International Committee for Standardization in Haematology to consider various aspects of quality control. The work was presented at symposia held at the XIII Congress of the International Society of Haematology and is edited by Professor G. Izak and Dr S. M. Lewis.

The first part of the book is devoted to various aspects of measuring haemoglobin concentration, and includes an account of the spectral characteristics of haemoglobin derivatives, reagents used in the cyanmethaemoglobin method, and the value of Ringbom curves in haemoglobinometry. The second part concerns the studies of standardization of serum iron and iron-binding capacity assays and includes results of inter-laboratory trials. The

third part is devoted to haemocytometry and includes an account of the chemical and physical aspects of electronic cell counting, electronic cell sizing; evaluation of various cell counting instruments and methods of quality control applicable to such instruments. The final part describes certain of the computer aspects of data processing in haematology, interfacing with general hospital systems and computer-aided differential diagnosis.

The title appears somewhat misleading, but this volume will prove of interest to all those concerned with standardization and quality control in haematological laboratories.

J. D. M. RICHARDS

Rays in Space

Cosmic Rays. By A. M. Hillas. (The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies: Selected Readings in Physics.) Pp. x+297. (Pergamon: Oxford and New York, August 1972.) £4.

IN spite of the vastness of the subject (or perhaps because of it) there have not been many books on cosmic rays and Dr Hillas's addition is very welcome. The author is an international authority on the analysis of complex air shower data with the object of determining primary characteristics of astrophysical interest and his ability in interpreting the many and varied phenomena encountered in cosmic rays shines through very clearly in this volume.

The subject is treated very largely in historical fashion and in fact half the book is devoted to reprints of some of the key papers which have marked turning points in the development of the subject. The theme is the development of ideas about the nature of the various particles encountered in the radiation, and their interactions, leading to an examination of the problem of the primaries, their origin and their relationship to other extra-terrestrial radiations.

This book, attractively produced, will be read with profit by physics undergraduates and must surely become prescribed reading for postgraduate students entering research fields in the space sciences in general.

A. W. WOLFENDALE

Addendum

IN the bibliography to the review of the *UFO Experience* (*Nature*, **239**, 529; 1972), the publisher was given as Henry Regnery: Chicago. The book is also published by Abelard-Schuman: London.

CORRESPONDENCE

Soviet Scientists

SIR,—This is the typescript of a message received by telephone from Professor Levich on November 26, 1972.

Open Letter to Academician Keldysh, President of the USSR Academy of Sciences:

Since 1724, when the Russian Academy of Sciences was founded, there has hardly been a situation like the present one. For nearly a year, the President of the Academy has been declining to see, or even to reply to the letters of, a group of scientists including professors and a corresponding member of the Academy. Therefore we are compelled to address you in this open letter.

We believe that as the official head of Soviet science you must, and that as a member of the Central Committee of the Communist Party you can, intervene actively in the tragic case of scientists who wish to repatriate to Israel and who are forcibly detained in this country under the pretext of "government interests" and "secrecy considerations". You will certainly agree that the progress of science cannot be achieved without freedom of conscience and freedom of moral convictions for scientists. The singling out of scientists and specialists into a discriminated category as compared to others cannot but arouse the concern and indignation of the world scientific community.

It is your duty to explain to the authorities that the forcible detention of scientists, and at the same time their persecution, is doing irreparable harm to the prestige of that body of scientists of which you are the leader. You know that, quite apart from written agreements and the formal fulfilment of exchange plans, spiritual cooperation among scientists of different countries is a significant reality; and it is this kind of cooperation which is being damaged, because the world scientific community cannot and will not remain indifferent to the persecution of its members. This is what actually does real harm to the government's interests.

As to the so-called secrecy, to begin with you will probably agree that, under existing Soviet conditions, those who are at present really aware of any important secrets will not apply for exit visas. As a scientist, you understand perfectly well that scientific progress is so rapid that scientific and technological secrets become obsolete within some two or three years.

Meanwhile, just as you, on your visit to the United States of America, were proclaiming a humane and reasonable approach to the problem of repatriation of Jewish scientists, the deputy Minister of the Interior, General Shumelin, publicly declared the time of detention of scientists on the grounds of secrecy to be up to twenty years. We ask you, Mr President: who determines the fate of scientists in the long run? You, the head of Soviet science or generals of the Ministry of Internal Affairs?

The agreement which has been recently concluded between the USSR and the USA has clearly shown that the exchange of scientific and technological information is much more important than the keeping of secrecy, even in such new and important areas as the cosmos. We wonder whether in this situation it is admissible to treat seriously the poor remnants of the allegedly secret but long obsolete information known to some of us.

The convincing proof that the so-called secrecy considerations are worthless is the fact that several scientists and specialists obtained exit visas this October who, only a few weeks before, were refused visas on "secrecy considerations". Explaining the situation more frankly, General Verein, the chief of OVIR of the USSR, who is responsible for questions concerning exit permits, on November 20 officially confirmed that it is preferable to let a scientist rot in this country than to enable him to work somewhere else; our worst predictions have obviously come true. We have already come to be considered as the inalienable property of the state.

Mr President, we suggest that you should publicly condemn such disgraceful treatment of those who dare to fight for their natural human rights; otherwise you inevitably become an associate of those who promote the above ideas. We suggest that you should secure the legally established procedure of repatriation for scientists to Israel, this procedure being public and based on reason and laws. We wonder, Mr President, whether you yourself will perceive it as your urgent moral duty so to do.

Signed by: Benjamin Levich, corresponding member of the Academy of Sciences of the USSR; Professor Alexander Lerner; Professor Vladimir Mash; Professor Jevsei Ratner; Professor Alexander Voronel.

Yours faithfully,

D. B. SPALDING

Imperial College,
London SW7 2BX

Cancer Research

SIR,—May I be allowed to correct two statements in the article in your issue of December 1 headed "Lord Zuckerman Defends His Position", in which you refer to remarks I am quoted as having made at a meeting of the British Association for Cancer Research.

The first is in the opening sentence, which says that I have advocated a "policy of parsimony in cancer research".

I have advocated no such policy, either for cancer research or for any other form of research. If such a policy has been mooted, it must have been in your original comment on the report I prepared for the Prime Minister (*Nature*, 240, 4; 1972), and to which you now refer again. As opposed to what appears in *Nature*, what I wrote is that a sudden increase in funds for cancer research could not be effectively used, whereas "a steady and substantial increase over the years would probably yield valuable results".

Your second error is the statement that I "launched a counter attack on the scientific community", which I accused of "narrow mindedness". I have levelled no such accusation, and since I have not been attacked, there was no need, even if I had wished to do so, for me to launch a counter attack. What I pointed out at the meeting was that applications for research grants are inevitably judged by assessors whose personal experience of what constitutes fruitful research necessarily influences, some would say prejudices, their judgment. There is, however, no sensible alternative to this normal procedure for dealing with applications for funds, and equally there is nothing new in my observation. It has been made time and time again, not only in comment on what the Americans call "peer review", but also on the review process whereby a scientific paper is judged worthy of publication. As those who are familiar with the history of science know, there have unfortunately been instances of reports of major scientific advances whose publication has been delayed for years, even decades, by what we would today call the editorial process. Instances can even be found in the cancer field. What I appealed for at the meeting at the Association for Cancer Research is that this fact be borne in mind when support is sought for what may seem, in the frame-

work of established and conventional thought, as way-out ideas.

Yours faithfully,

ZUCKERMAN

*The Shooting Box,
Burnham Thorpe,
Kings Lynn,
Norfolk*

Cancer Research

SIR,—Your correspondent who wrote "Lord Zuckerman Defends his Position" (*Nature*, 240, 247; 1972) fails to distinguish between a "polite" and a "deafening" silence. Sir David Smithers was, of course, wrong in claiming that supracellular biologists have made a greater contribution to the progress of cancer therapy than molecular biologists. The most important new advances in therapy in the past decade have stemmed from the development of chemotherapeutic agents. All the anti-metabolites, such as mercaptopurine and fluorodeoxyuridine, antibiotics such as actinomycin D and specific inhibitors of nucleic acid metabolism, such as methotrexate, have emerged from biochemical research. Most eukaryotic molecular biologists also consider tissue culture one of their tools and this has revealed other cytotoxic substances such as vincalcalcin and asparaginase. The truth is, of course, that Sir David was being pious in making the reasonable point that we should not adopt an unbalanced attitude towards cancer research: Lord Zuckerman was making precisely the same point. He has, by the way, been misquoted in your report. He has not recommended a parsimonious attitude to cancer research. What he has stated is that, with certain exceptions, he sees no case for "a sudden increase in funds" but recommends "a steady and substantial increase over the years" (Zuckerman report, page 12).

It would be a pity if we in Britain buried our heads in the sand in relation to cancer research. The facts are reasonably well established. By the application of all the best radiotherapy, chemotherapy and early diagnostic methods at our disposal, we could improve the cancer statistics by only a few (5–10) per cent. By appropriate preventive measures (which would require the banning of smoking in this country), we would do very much better and might achieve a 35 per cent improvement. In ideal circumstances, however, at least 60 per cent of those at present destined to die of cancer would still do so, half of them before the age of 60. There can be no doubt that advances beyond that will depend

entirely on innovative research. I would not find it profitable to argue whether subcellular or supracellular approaches are likely to prove more rewarding in the long run. This distinction in cancer research is entirely artificial and divisive and I doubt if we will get far unless we support both. This is what I understood to be the message from both Sir David and Lord Zuckerman.

Yours faithfully,

JOHN PAUL

*Royal Beatson Memorial Hospital,
The Beatson Institute for Cancer
Research,
132 Hill Street,
Glasgow G3 6UD*

Errata

IN the article "The First Fossil Record of Caecilian Amphibians" by Richard Estes and Marvalee H. Wake (*Nature*, 239, 228; 1972), the caption to Fig. 1 should read as follows: Vertebrae of fossil and recent caecilians: *a-e*, *Dermophis mexicanus*, MVZ (University of California Museum of Vertebrate Zoology) 98255, posterior trunk vertebra; *f-j*, *Apodops pricei*, n. gen., n. sp., DGM 551, the same; *k-o*, *Geotrypetes seraphinii*, MVZ 98253, the same. From top to bottom, views are anterior, posterior, left lateral, ventral and dorsal. Anterior is to the left in *c-e*, *h-j* and *m-o*. Parapophyses and part of posterior border of neural arch are restored in *h-j*. Magnification line at left refers to *a-j*; at right to *k-o*.

IN the article "Methadone Induced Mortality as a Function of the Circadian Cycle" by Robert H. Lenox and Thomas

W. Frazier (*Nature*, 239, 397; 1972), the sentence beginning on line 10 of paragraph 7 should read: "The most sensitive portion of the circadian cycle appears to occur at the period of transition from rest to activity, when a number of life systems are beginning to accelerate their activities from their minimal resting levels". The address of Robert H. Lenox is Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington DC 20012.

IN the article "Bode's Law and the Missing Planet" by M. W. Ovenden (*Nature*, 239, 508; 1972), the second sentence of the penultimate paragraph should read: "From the point of view of the dynamical arguments presented here, it is probably true that A might always have been in the form of a ring".

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.

The direct postal price per subscription is:

12 MONTHS (52 issues per title)

	Surface mail UK and worldwide	U.S.A. and Canada
Nature (Friday)	£16	\$48
Nature + Nature Physical Science	£24	\$83
Nature + Nature New Biology	£24	\$83
All three editions	£29.50	\$108
Annual Index	£1	\$3

(Charge for delivery by air mail on application)

Editorial, Advertising and Publishing Offices of NATURE

MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2R 3LF
Telex 262024

MACMILLAN JOURNALS LIMITED
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number: 202-737 2355. Telex 64280

International Advertisement Manager
PETER R. KAVANAGH
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Numbers: UK 01-836 6633. USA 202-737 2355

Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS RG21 2XS
Telephone Number: Basingstoke 29242

Classified advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone Number: 01-242 6264/01-405 4743
Telegrams: Textualist London WC2A 2ED

Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, December 22 1972